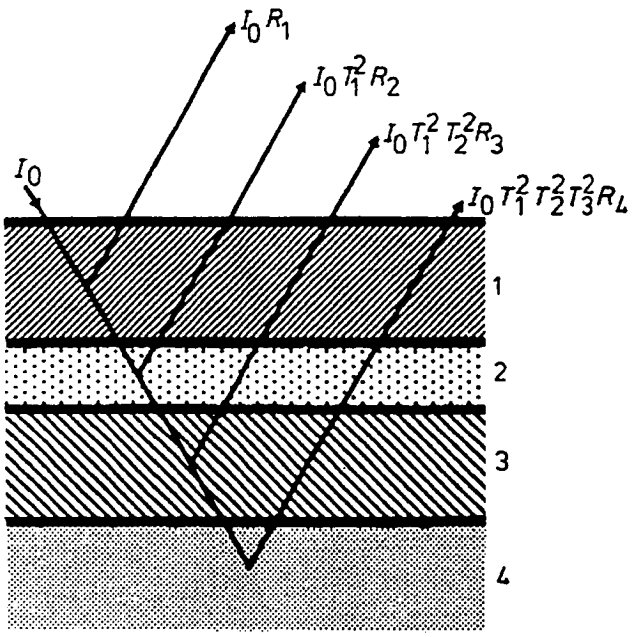
Reflectance Spectrophotometry in the Assessment of Radiation Erythema.



C S HAMILTON

University of Tasmania
Faculty of Medicine and Pharmacy

REFLECTANCE SPECTROPHOTOMETRY IN THE ASSESSMENT OF RADIATION ERYTHEMA

Chris
C S HAMILTON
B Med Sci, MBBS (University of Tasmania 1978, 1981)
FRACR 1988

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To Mary, Benjamin and William
Newcastle, Nov 1995.

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DECLARATION

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The patients enrolled in the FLDR and FHDR studies were cared for by Dr C Hamilton, Prof J Denham and Dr D Joseph. All clinical reaction scoring in these patients and for the artificial erythema studies was performed by Dr C Hamilton or Prof J Denham in co-operation with the clinical nurse on our research team. All FLDR therapeutic irradiation was delivered by Ms Sally Simpson, reflectance measurements by Ms Maree O'Brien / Ms Suzanne Wright and dosimetric measurements performed by the Physics team at the Mater Hospital. Data presented in this thesis has been analysed exclusively by myself, apart from ABest procedures and generalised linear regression modelling (Dr T Kron and Mr F Tuyl respectively).

This work was collaboratively supervised by Prof J Denham over the entire five year period of the study. My academic supervisor was Prof HK Muller,(Professor of Pathology, University of Tasmania, Faculty of Medicine and Pharmacy, Department of Pathology, Clinical School Royal Hobart Hospital, 43 Collins Street, Hobart, Tasmania). Papers arising from this work (published and in press) are included in the appendix.

This thesis contains no material which has been submitted or accepted for any other degree or diploma in a tertiary institution. This thesis contains original work (apart from contributions as above) and all other published work is fully referenced.

Signed

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PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

Cooper SG, Cardew AP, Ferguson S, Williams AR, Joseph DJ, Hamilton CS and Denham JW. (1990) Low dose rate teletherapy using a telecaesium 137 unit; radiobiological, physical and clinical considerations. *Australasian Radiology* 14, 241-246

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Hamilton CS, Simpson SA, Ferguson S, Ostwald PM, Hsu W, O'Brien MY, Denham JW. (1993) Low dose rate teletherapy and tumour response. *Australasian Radiology* 37 210-212

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PRESENTATIONS AT MEETINGS

Denham JW, Joseph DJ, Hamilton CS, Simpson SA, O'Brien MY, Ostwald PM, Hsu W, Ferguson S. (October 1994) Preliminary results from the low dose rate teletherapy project conducted in Newcastle Australia: Acute mucosal reactions. *Royal Australasian College of Radiology*, Christchurch New Zealand

Hamilton CS, Denham JW, Simpson SA, O'Brien MY, Kron T. (October 1994) The response of human skin to fractionated low dose rate radiotherapy. *Royal Australasian College of Radiology*, Christchurch New Zealand

Denham JW, Joseph DJ, Hamilton CS, Simpson SA, O'Brien MY, Ostwald PM, Hsu W, Ferguson S. (September 1994) Preliminary results from the low dose rate teletherapy project conducted in Newcastle Australia. 1: Acute skin reactions. *ESTRO*, Granada Spain

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Joseph DJ, Hamilton CS and Denham JW. (1990) Low dose rate teletherapy - effects on oropharyngeal mucosa and skin over a range of dose rates. *ESTRO*, Montecatini

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ABSTRACT

This thesis describes the dose response relationships, qualitative and quantitative observations of cutaneous erythema in 236 patients treated on three prospective clinical radiobiological studies, utilising Fractionated Low Dose Rate (FLDR) and Fractionated High Dose Rates (FHDR) external beam radiotherapy.

- Patients with incurable, locally advanced head and neck cancer and bone and soft tissue metastases from a variety of other solid malignancies, were treated on a modified Caesium 137 teletherapy unit , at dose rates ranging from 0.8 to 8.2 Gy/hr in 10 fractions over 12 to 14 days (FLDR).

- Patients with bony and soft tissue metastasis from a variety of solid malignancies were treated on a Clinac 1800 (6 MV photons), utilising four fractionation schedules (5, 10, 12 and 20) over 5 to 35 days (FHDR).

- Patients with localised carcinoma of the prostate were treated on a Clinac 1800 (6 MV photons), in 30 - 32 fractions over 45 to 55 days (FHDR).

Skin reactions were quantitatively assessed using a reflectance spectrophotometer and graded qualitatively using a modified EORTC/RTOG grading scale (FLDR). Qualitative skin scoring was unreliable and subject to considerable inter- and intra-observer variation. Qualitative scores were over estimated (relative to reflectance observations) in female patients and at non-UV exposed anatomical sites. Pre-treatment reflectance readings were significantly higher in male patients and in anatomical sites which had previously heavy UV exposure. Pronounced dips in erythema readings during the second week of therapy and "reciprocal vicinity" effects adjacent to the treatment field, undetected by the naked eye, were observed in a subset of patients. Peak erythema values were found to provide the best measure of radiation effect. Peak erythema was found to depend on biologically effective skin dose, patient age, sex and treatment site. Considerable inter- and intra-patient heterogeneity (both before and during treatment) was demonstrated. No dose-rate effect was seen in the FLDR group and a negative dose response relationship was demonstrated for 0.8 Gy/hr patients. FHDR data was under-predicted by the Linear Quadratic model at doses less than 1.5 Gy/fraction. This finding is potentially explained by the "Induced Repair Model" of Joiner et al. For doses above 2 Gy/fraction a reasonable fit was obtained giving an α/β ratio in the range of 4-8 and a repair half time of 0.05- 0.15 hours. This short $t_{1/2}$ value is not consistent with other reports and may reflect multi-component cellular repair processes. This study also suggests that radiation-induced erythema is not exclusively related to basal cell kill.

KEYWORDS: Skin, Erythema, Radiotherapy, Low Dose Rate, High Dose Rate, Reflectance Spectrophotometry, UV radiation, α/β ratio, Repair Kinetics.

LIST OF ABBREVIATIONS

$^{99}Tc^{M}$	- Metastable Technetium-99
AUC	- Area Under Curve
BED	- Biologically Effective Dose
CIE	- Commission Internationale de l'Eclairage
Cs_{137}	- Caesium 137
D_0	- Slope of the initial portion of cell survival curve
D6	- Duration of reaction 6 erythema units above baseline
D12	- Duration of reaction 12 erythema units above baseline
D18	- Duration of reaction 18 erythema units above baseline
ED_{50}	- Expected Dose for 50% Isoeffect
EI	- Erythema Index
EORTC	- European Organisation for Research on the Treatment of Cancer
EPU	- Epidermal Proliferative Unit
ERD	- Extrapolated Response Dose
FHDR	- Fractionated High Dose Rate Radiotherapy
FLDR	- Fractionated Low Dose Rate Radiotherapy
Gy	- Gray
ICRU	- International Commission for Radiation Units and Measurement
IR	- Incomplete Repair
kV	- kilovolts
LED	- Light Emitting Diode
LI	- Labelling Index
LiF	- Lithium Fluoride
LIR	- Logarithm of the Inverse Reflectance
LQ	- Linear Quadratic
MED	- Minimal Erythema Dose
nm	- nanometres
OI	- Oxygenation Index
P	- Probability
RE	- Relative Effect
RTOG	- Radiation Therapy Oncology Group
SD	- Standard Deviation
SE	- Standard Error
SF_2	- Surviving Fraction after 2 Gy
SLD	- Sub-Lethal Damage
TLD	- Thermoluminescence Dosimetry

UVA - Ultraviolet radiation, A band

UVB - Ultraviolet radiation, B band

UVC - Ultraviolet radiation, C band

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CHAPTER I BACKGROUND TO STUDY

1 INTRODUCTION AND SCOPE OF STUDY

Radiotherapists and research scientists have been observing the effects of ionizing radiation on mammalian skin for the better part of the twentieth century. Quantification and qualification of acute radiation effects in mammalian skin systems, such as the mouse, rat and pig, have received extensive attention in the radiobiological literature, however, apart from the work of Turesson, little systematic scientific study of the radiation response of human skin has occurred. (Turesson & Thames 1989; Reisner 1933; Overgaard et al. 1985; Dutreix et al. 1973; Bentzen et al. 1987; Russell et al. 1994) The reasons for this are diverse, however principally revolve around the intrusion of clinical and medical demands on both the patient and radiotherapist in the setting of ionizing radiation delivered for the primary purpose of treating malignant disease. Assessment of cutaneous radiation erythema at both high and low dose rates offers the opportunity for considerable insights to be gained into fractionation sensitivity and repair characteristics without having to resort to prescribing a wide range of fraction sizes. Ideally in a study such as this, one would select a cellular endpoint which reflects basal cell kill in response to irradiation. However, the invasiveness of biopsy procedures required to do this are significant. The relationship between basal cell kill and a physiological endpoint such as erythema, in the human setting remains unknown and traditional assumptions that erythema is monotonously related to basal cell kill (at least over a limited dose range) have not been rigorously tested.

Mammalian experimental radiobiology has evolved a series of functional end-points which are readily applied to a variety of organ systems; such as respiratory rate and fore-leg paralysis in the mouse, for lung damage and spinal cord damage respectively. Arriving at similar functional end-points for acute radiation epidermal erythema, is not so easy a task. Semi-quantitative descriptive scales have been employed, which produce their own special set of mathematical, conceptual and analytical problems. Thus a quantitative measure of cutaneous erythema, such as is provided by reflectance spectrophotometry, has been and remains a potentially attractive tool for evaluating acute radiation effects in the skin. This project describes the application, results, interpretations and pitfalls of this technique in three prospective clinical radiobiological studies.

2 CUTANEOUS PHOTOBIOLOGY

It is clear that the human organism requires certain exposure to natural sunlight, for instance, the ultraviolet photo-chemistry of vitamin D and the visible light photo-chemistry of the retina, to allow visual perception. However, it is equally clear that excessive solar irradiation of the skin causes immediate inflammation and altered vascular pathophysiology in the short term and in the long term, actinic damage leading to malignancy. Human skin has developed peculiar optical and chemical mechanisms which protect against ultraviolet radiation and in some instances regulatory feedback mechanisms for increasing the degree of protection. Photobiology is the study of the interaction of ultraviolet visible and near infra-red radiation with various bio-molecules and the sequelae of that interaction.

2.1 **Ultraviolet Effects**

Visible light occupies a range from approximately 400 - 700 nanometres in the electromagnetic spectrum. Tissue interactions over this range do not cause any photo-chemical events that result in recognisable tissue alteration or damage. (Figure 1) This is not the case with ultraviolet radiation, whose deleterious effects in overdosage are well described. (Soter 1990; Biochemistry and Physiology of the Skin 1983; Dermatology in General Medicine 1987; Textbook of Dermatology 1987). Photobiologists generally divide the ultraviolet spectrum into three portions UVA, UVB and UVC in order of decreasing wavelength. Radiation in the UVC band causes erythema of normal skin and photokeratitis very efficiently. UVB radiation reaches the earth in relatively smaller quantities but is very efficient in causing erythema in human skin. Epidemiologic evidence suggest that solar UVB causes skin cancer in man. UVA radiation is both melanogenic and erythmogenic, however, is not as efficient as either of the other two ultraviolet bands. Both the phenomenon of erythema and melanin production in human skin alter the optical properties of the dermis in such a manner as to minimise further optical damage.

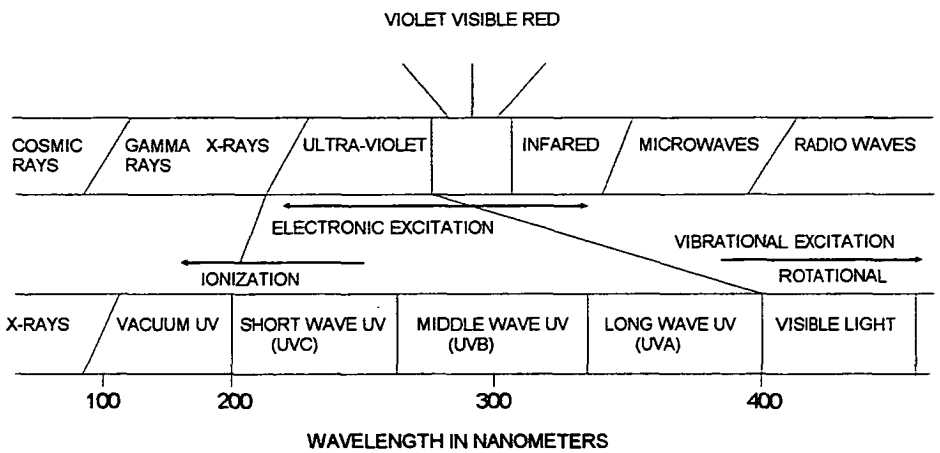


Fig. 1 Electromagnetic spectrum with expanded scale of ultraviolet radiation

2.2 Optical Properties of Human Skin

Any given incident radiation beam on human skin may suffer one of two basic interactions:

i) scattering
ii) absorption

Absorption involves the transfer of energy from an incident photon into the absorbing material, producing an excited state in the absorbing molecules. In optical and UV wavelengths this excited state does not result in ionisation of the molecule. Scattering on the other hand does not involve the transfer of energy to the absorbing medium but merely alters the direction of propagation of the radiation. Thus the reflection of light from a mirror represents the phenomenon of regular or specular scattering. Scattering occurs whenever radiation encounters boundaries between media of different refractive indices. When radiation strikes the skin, a portion is scattered back to the environment, a further portion is absorbed in the various layers of the skin and the remainder is transmitted inwards through successive cellular layers until the energy of the incident beam has been dissipated. (Figure 2) Because the epidermis is thin and primarily cellular as opposed to fibrous, optical scattering other than the diffusion occurring at the skin/air interface does not appear to be of major importance in determining its transmission of UVA visible and near infra-red radiation. The epidermis can be approximated in the first instance to mimic the optical properties of an unpolished glass absorption filter, underneath which are various chromophores and further layers.

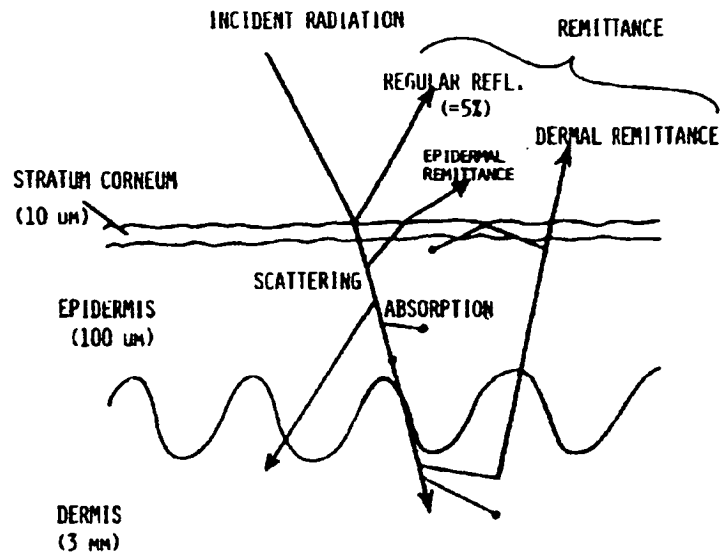


Fig 2. Schematic diagram of optical pathways in skin.

Scattering within the dermis is an inverse function of wavelength (Anderson & Parrish 1980). Because of this approximately 50% of UVA radiation which penetrates fair caucasian epidermis is largely attenuated within the first 50 micrometres of the papillary dermis, whereas radiation in the optical range (eg. green at 500 nanometres) may penetrate several hundred microns and red and near red infra-radiation several millimetres more. (Figure 3) It is this phenomenon, combined with the minimal reflectance of the epidermis, which is responsible for the major reflected optical properties of human skin. This applies both to subjectively perceived colour, (ie the human retina) and objective measures of reflectance in the optical range. The colour of healthy human skin is determined largely by the quantity and degree of oxygenation of blood in the dermis and the presence or absence of the brown/black epidermal pigment melanin. These principles may also account for the appearance of the so called "blue naevus" due to dermal melanin. (Anderson and Parish 1981) In this instance, shorter wavelength light, ie blue light experiences a shorter path length from relatively superficial layers of the dermis and hence comparatively less absorption by melanin than do longer wavelengths. Because human colour perception is largely comparative, the relative depletion of reflected longer wavelengths relative to shorter wavelengths account for the appearance of the "blue naevus".

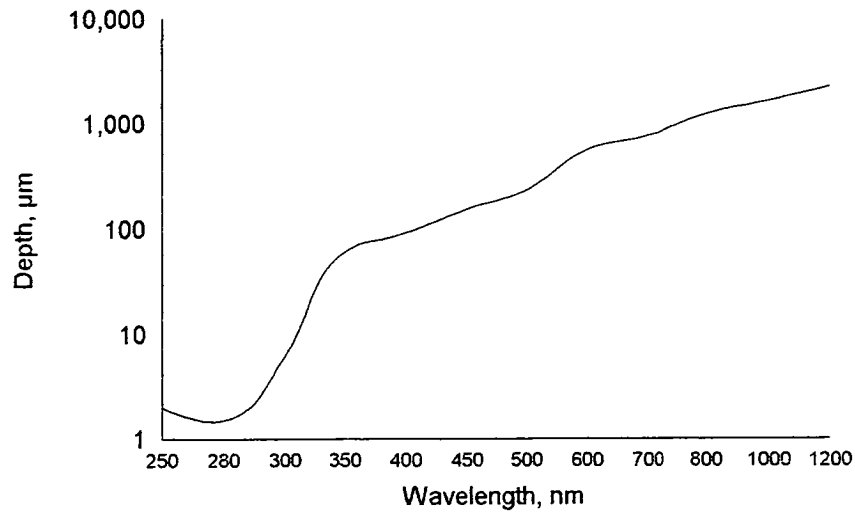


Fig. 3 Approximate depth for penetration of optical radiation in fair caucasian skin to a value of 1/e (37%) of the incident energy density.

Despite the multitude of factors affecting reflectance, the properties of human skin even across different races are surprisingly constant. Regardless of melanin content, the 250-300 nanometres region is strongly absorbed (Anderson & Parish 1981). The epidermal transmittance of 330-1300 nanometres radiation is high for fair Caucasian skin but decreases markedly at progressively shorter wavelengths as the melanin content is increased. Haemoglobin and deoxyhaemoglobin absorb strongly in the blue and green visible regions. Between the infra-red absorption bands of water and the green visible absorption band of haemoglobin lies the spectral region of greatest penetration. Because of the increased dermal scattering and optical absorption in-vivo for wavelengths less than 600 nanometres, and the high optical absorption of wavelengths longer than about 1300 nanometres by water, an optical "window" exists in skin and most other soft tissues in the region of 600-1300 nanometres. Figure 3 displays the calculated attenuation levels of various wavelengths of light in human skin to levels of 1/e of their incident intensity. (Anderson and Parish 1981). Obvious from Figure 3 is the observation that both ultraviolet and visible radiation reach the level of cutaneous blood vessels. Thus endothelial cells, the blood elements, lymphatics and neural processes passing through the skin may be directly or indirectly affected by incident light in the ultraviolet and visible range and similarly may also contribute to the intensity and wavelengths of reflected light.

3 THE ASSESSMENT OF SKIN COLOUR

3.1 The CIE System

The exact perception of skin colour and grading on an arbitrarily defined scale by trained human observers is a complex task. The perception of subtle colour and surface changes by the human eye, along with all the other familiar causes of inter and intra-observer variation have meant that for some fifty years imperfect attempts have been made to quantify skin colour. Surface colour may be quantified when using the CIE System, Commission Internationale de l'Eclairage (1986), (Nimeroff 1964). Other workers have subsequently employed this system for the evaluation of erythema following local irritants and ionising radiation (Serup & Agner, 1990; Lebesque 1992 personal communication). The CIE System is adapted to the non-linear colour perception of the human eye. In this model, a colour is expressed on a 3-dimensional co-ordinate system; along the A axis (green/red), B axis (yellow/blue) and L axis (brightness). (Figure 4). The CIE System is appropriate when simple colour matching is required eg. in paint or fabric manufacture, but does not give any information about the substances actually responsible for absorption and reflection. The CIE system and indeed any other reflectance system does not take into account the subtle physiological changes which maybe only observable by the human eye, following noxious insults to the skin. (eg early dry desquamation, skin oedema, or peri-follicular erythema).

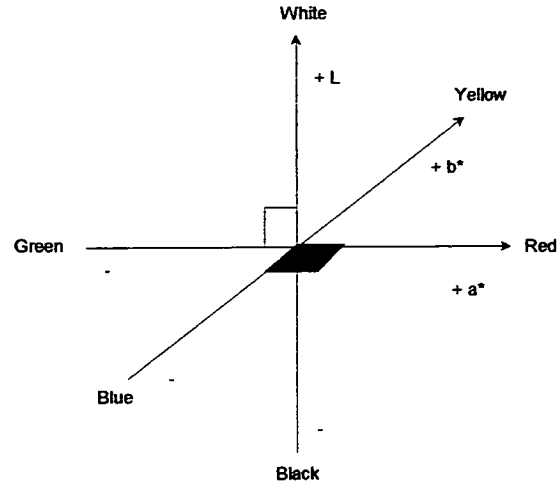


Fig. 4 The Commission International de l'Eclairage (CIE) colour system. This system is a three-dimensional coordinate system with an a* -axis, a b* -axis and an L -axis.

3.2 Reflectance Methods

Edwards and Duntley (1939), first quantified skin colour some fifty years ago by measuring visible light reflectance of the skin with a spectrophotometer. Since that time the principles of reflectance spectrophotometry have been employed in a range of anthropological and genetic studies (Green and Martin 1990; Westerhoff et al. 1990). Reflectance spectrophotometry has found its principle use in the area of quantification of ultraviolet induced skin erythema. (Chu et al. 1960; Sayre et al. 1966; Kollias & Baqer 1988; Farr & Diffey 1984; Wan et al. 1983; Whitman et al. 1985; Anderson & Bjerring 1990). Reflectance spectrophotometry has also been employed in the evaluation of colour changes in the behaviour of port wine stains (Lanigan & Cotterill 1988), therapy for psoriasis (Ryatt et al. 1983), tumour blood flow (Feather et al. 1988) and assessment of patch test responses (Mendelow et al. 1986; Malm and Tonnquist 1988). The development of the science of photomedicine has stimulated the production of a number of reflectance instruments in the response to the need to precisely characterise the inflammatory response seen in a variety of dermatological conditions, since the visual assessment of erythema does not fulfil this criteria, even when aided by various comparative visual grading scales. The human eye is an excellent device for sensing a quantal event but is inadequate in delineating graded differences of erythema or changes in time. (Daniels & Imbrie 1958)

3.3 Laser - Doppler Methods

Another method which has been used for the evaluation of erythema has been laser-Doppler blood flow measurements. This method depends on light reflectance from a helium-neon laser beam. The reflected light is detected by two separate detectors and the doppler shift measures the total movement of particles in the skin, allowing quantification of skin blood flow. This method has proved sensitive and valuable in the evaluation of UV induced erythema. (Anderson & Bjerring 1990; Weiss et al. 1992; Kimura et al. 1988; Serup & Agner 1990; Duteil et al. 1990). Laser-Doppler measurements have also been reported by Simonen et al, in the evaluation of radiotherapy induces skin reactions post-mastectomy and in pig skin. (Nicklen et al. 1994; Simonen et al. 1994)

3.4 Comparison of Reflectance Spectrophotometry to Other Methods of Skin Colour Assessment

There have been limited studies on the parallel evaluation of both subjective assessment of erythema and objective assessment using reflectance spectrophotometry. Wan et al. 1983, studied six fair skinned caucasian volunteers who were exposed to sample doses of broadband UVB and assessed using a reflectance spectrophotometer which utilised three wavelengths 544, 577 and 610 nanometres. The investigators also subjectively graded erythema according to the following scale:

0	- no erythema
trace	- minimally perceptible erythema
1+	- minimal erythema with definite borders
2+	- more pronounced erythema
3+	- erythema with oedema

At corresponding times they derived a blood volume parameter related to the superficial plexus based on reflectance values. As shown in Table 1, they demonstrated that each subjective grade of erythema corresponded to a broad range of blood volumes and vice versa. That is, a wide overlap in blood volumes was present between consecutive grades. They also noted that the threshold erythema which determines the minimum erythema dose (MED) and which should be accurately sensed visually, as a sharp threshold observation, also showed a wide variation in measured blood volumes.

TABLE 1

Subjective			Objective	
Grading	Definition	Number of Assessments	Range of Cutaneous Blood x 10^4 (ml/cm^2)	Mean ± S.D. x 10^4 (ml/cm^2)
0	No erythema	80	33.7 - 130.4	75.9 ± 24.4
Trace	Minimally perceptible erythema	23	71.6 - 133.3	104.8 ± 21.3
1+	Minimal erythema with definite borders	30	84.5 - 203.4	120.8 ± 28.4
2+	More pronounced erythema	31	89.8 - 215.4	160.1 ± 35.3
3+	Erythema with oedema	55	129.1 - 253.4	189.5 ± 31.7

Table 1 Ultraviolet-B (290-320 nm) induced erythema; comparison of subjective and objective assessment. (Wan et al. 1983)

The authors concluded that reflectance spectrophotometry was a far more sensitive method than visual grading and appeared to relate to UV dosimetry in a far more consistent fashion.

Anderson and Bjerring (1990), performed a similar study with nine volunteers exposed to a combination of UVA and UVB irradiation. They divided their clinical evaluation of erythema into four grades, and likewise found reflectance spectrophotometry to be suitable for detecting minor degrees of erythema which were linearly related to the reflectance score over the wavelength 540-575 nanometres. They also employed a Laser-Doppler blood flow meter in this study and demonstrated that reflectance spectrophotometry showed a higher sensitivity for the detection of early erythema than the Laser-Doppler method. Blood flow parameters with the erythema score, however, indicated that the Laser-Doppler blood flow measurements paralleled the dose response curve for the erythema index.

4 LIGHT REFLECTANCE THEORY

4.1 Reflectance and Transmission Cells

The theory of reflectance spectrophotometry shares many of the physical principles associated with conventional transmission spectrophotometry. This is a technique which is widely used particularly in medicine to measure the concentration of an absorbing, non scattering pigment in solution. The Beer-Lambert law of light transmission is given as equation 1:

$$I = I_0 e^{-(\mu c x)} \qquad (1)$$

where μ is the specific absorption coefficient of the pigment to be measured. The unknown quantity is C the pigment concentration. It can be simply shown that C is proportional to the natural log of 1/T, were T is the transmission factor ($T = I/I_0$). If we consider the situation where a perfectly reflecting surface, for example, a mirror is placed at the exit wall of the transmission cell we now have a simple reflectance cell, (Figure 5) in which the path length of the light is now 2x rather than x. In this situation however, C is now proportional to the natural logarithm of 1/R where R is the measured reflectance (I/I_0). Most authors refer to this expression as the Logarithm of the Inverse Reflectance (LIR) (Dawson et al. 1980; Wan et al. 1983; Anderson & Bjerring 1990).

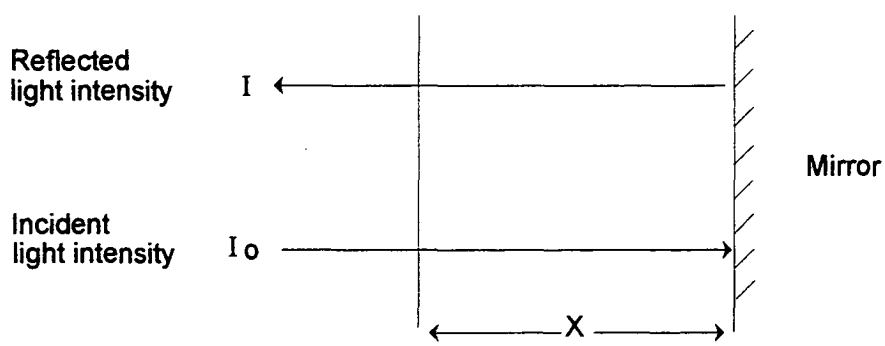


Fig 5. A simple reflectance cell.

Dawson et al. (1980) have developed a comprehensive mathematical model for the dermis and epidermis based on the Kubelka-Munk Theory of Light Transmission which simplifies to the following expression given certain assumptions. The expression is equation 2:

$$I = I_0(R_1 + T_1^2R_2 + T_1^2R_2 + T_1^2T_3^2R_3 + T_1^2T_2^2T_3^2R_4) \qquad \textbf{(2)}$$

Where I is the intensity of light reflected from the layered structure and I_0 is the intensity of the incident light (Figure 6)

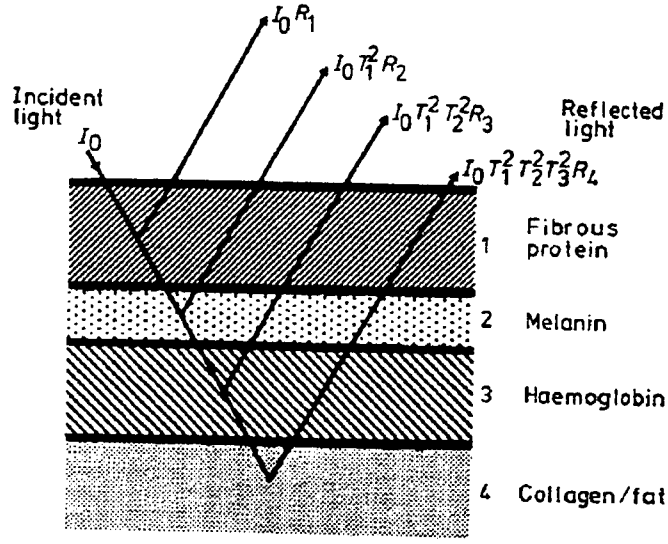


Fig 6. Simplified model of the layered structure of skin. I_0 = intensity of incident light, R = coefficient of reflection, T = coefficient of transmission. (Dawson et al. 1980)

In considering this model of skin as a reflectance cell we assume that:

i) the epidermis (neglecting its melanin content) neither absorbs nor scatters light.
ii) the blood and melanin pigments absorb but do not scatter light.
iii) the collagen fibres of the dermis (highly scattering but low absorption) act as a diffusely reflecting surface.
iv) the interface between layers does not modify the transmittance or reflectance of light by the system as a whole.

4.2 Application of the Model

The proposed theoretical model may be applied to non-pigmented skin in-vivo, by assuming that R1, R2 and R3 are all much less than 1. In this case the reflectance R of the skin approximates to equation 3:

$$R = I/I_0 = T_1^2 T_2^2 T_3^2 R_4 \qquad \textbf{(3)}$$

If the inverse of the reflectance (equation 3) is expressed in logarithmic form (equation 4) and plotted against wavelength, the resulting curve corresponds to the composite absorbance spectrum of the upper layers minus the logarithm of the reflectance function of the lowest layer (equation 5). If we let L = lg (1/R) then:

$$L = -\lg T_1^2 - \lg T_2^2 - \lg T_3^2 - \lg R_4 \qquad \textbf{(4)}$$

By substitution for T to exp(-Kd)

$$L = -\lg \exp(-2k_1 d) - \lg \exp(-2k_2 d) - \lg \exp(-2k_3 d) - \lg R_4$$

Let

$$A = 2\ kd\ \lg e$$

Then equation 5:

$$L = A_1 + A_2 + A_3 - \lg R_4 \qquad \textbf{(5)}$$

where the terms A_1, A_2 and A_3 correspond to the absorbances of the constituent layers, that is, layer 1 - fibrous protein, layer 2 - melanin and layer 3 haemoglobin, respectively, while R_4 is the reflection coefficient of the basal, collagen and fat layer. L is the logarithm of the inverse reflectance (properly abbreviated as LIR). This summation of absorbances implies that it may be possible to separate and quantify the contribution of each of the major components of skin colour. In order to make use of this theory Dawson and associates have drawn on the work of others and performed a series of verification experiments themselves which justify the assumptions given above.

4.3 Justification of The Model

The observations of Findlay, (1970) on the optical properties of connective tissue may be used to test the validity of the proposed model. Findlay's experiments consisted of progressively incrementing layers of neonatal dura mater over a non-reflecting black tile. He plotted reflectance over the entire optical wavelength and demonstrated essentially that connective tissue reflects more blue light and transmits more red. The equation used by Dawson et al. (1980), derived from the Kubelka-Munk Theory, agreed very closely with Findlay's experimentally derived curve. Since it was known that the intensity of reflectance from human skin is principally dependant on three absorbing pigments, oxyhaemoglobin, de-oxyhaemoglobin and melanin, Dawson et al. designed a reflectance instrument which utilised a continuous rather than discontinuous selection of wavelengths over the entire optical range. This is in contrast to other contemporary instruments (Diffey et al. 1984) which utilise a two wavelength skin reflectance approach for quantifying ultraviolet induced erythema.

Figures 7 and 8 indicate the complexities of reflectance for a variety of in-vitro and in-vivo pigments showing the range of overlap for the absorption peaks of each. These figures demonstrate the necessity to utilise a reflectance instrument which measures over a continuous range of wavelengths to allow for compensation of values due to the presence of multiple pigments in varying concentrations in the skin under consideration.

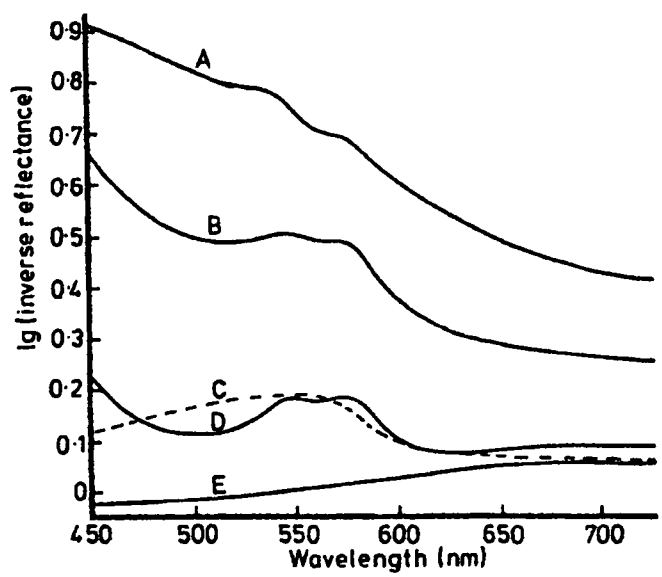


Fig 7. LIR and spectra of *in vivo* human skin. A: dark negroid. B: light negroid. C: pink tile. D: Caucasian.E: glazed white tile.

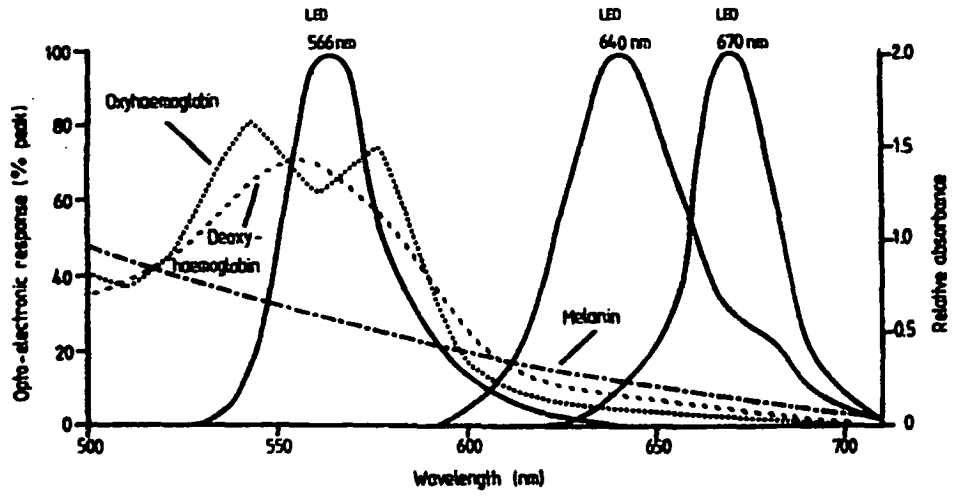


Fig 8. Opto-electronic response of Haemelometer together with *in vitro* absorbance spectra of oxyhaemoglobin, deoxyhaemoglobin and melanin. (Dawson et al. 1980).

4.3.1 Effects of Haemoglobin

The colour of non-pigmented skin is determined largely by the quantity of blood in the dermis, particularly the sub-papillary venous plexus (Lewis 1926). Dawson et al. (1980) performed a series of experiments in-vivo and in-vitro to determine the effect of blood in the sub-papillary venous plexus. They developed a parameter H, (Haemoglobin Index) which was based on differences between 3 isobestic points (544, 527.5 and 573 nm.) in the LIR spectra and which was found to be strongly correlated with erythema and negative for skin blanching. This index was then tested against an accurately quantified, increasing scale of colouring pigments which were similar to healthy caucasian skin. The rank correlation coefficient between the pigment concentration and haemoglobin index had an average value of 0.97. The haemoglobin index was significantly more accurate than subjective rating by separate individuals who had average correlation coefficients of 0.32 - 0.37. They also tested the instrument with UV induced erythema and adrenaline induced blanching and again found excellent sensitivity with moderate to good observer-based correlation (average correlation coefficient 0.87).

4.3.2 Effects of Melanin

By subtracting the caucasian skin reflectance curves from the curves derived from a group of negro subjects, a spectral response may be obtained, which is attributable to melanin alone. Dawson and colleagues (1980) used this difference, which is quite linear over the measured optical wavelength range to derive a pigmentation index, which is proportional to the melanin concentration in the skin of the subject.

4.3.3 Effects of Connective Tissue

In a similar manner, the absorption and scattering properties of light by in-vivo fibrous protein, connective tissue and fat may be determined by subtracting the haemoglobin absorbancies from the experimentally determined reflectance spectrum of vitiligious skin. This result is almost equivalent to zero and implies that fibrous protein, collagen and fat do not contribute significantly to the LIR spectrum of skin and that the diffuse reflectance of the lowest layer R_4 closely approximates one.

4.3.4 Other Optical Factors

Hajizadeh-Saffar et al. (1990) performed further verification experiments on the original Dawson instrument. They measured and corrected for the intensity of light scattered at the skin surface. As superficially scattered light has not penetrated to the chromophore containing layers of the skin, it contains no information about the absorbing pigments. They developed a method of eliminating air-skin interface reflectance from the measurements, by means of crossed polarisers incorporated into the reflectance probe. They were also able to demonstrate that there was a minor difference in the effective optical path length for suspensions of whole cells containing haemoglobin rather than simple solutions of extra-cellular haemoglobin. These corrections were also incorporated in their revised reflectance unit.

5 RELEVANT STRUCTURE AND FUNCTION OF HUMAN SKIN

The human integument on average weighs approximately 2.1kg and has a surface area of approximately $2m^2$. Its primary function is to provide a physical barrier against environmental hazards. These might include water and electrolyte loss, protection from atmospheric factors including ultraviolet light, chemicals, physical protection as well as its important thermo-regulatory

function. Specialised skin appendages are important for sensory functions, excretion, milk production and production of pheromones. The epidermis is composed of tightly compacted layers of dead cells which are packed with a specialised protein; keratin. Below this is the loosely packed elastic and flexible connective tissue, the dermis. At the junction of the two there is a specialised membrane, the basement membrane. Throughout the dermis are specialised regions of skin the most important of which is the hair follicle which consists of an infolding of the epidermis. Other accessory organs in the integument include the paired multi-lobed sebaceous glands associated with each hair follicle and eccrine and apocrine sweat glands. Other specialised cellular structures include the Langerhans cell and melanocytes.

5.1 Epidermal Structure and Cell Kinetics

5.1.1 Structure

The stratified keratinising epithelium comprising the epidermis varies significantly in thickness across the body with unexposed sites such as those on the abdomen, thigh and axilla having the thinnest epidermis and soles and palm the thickest and most complex structurally (range 1-3mm). In many sites throughout the body, the epidermis invaginates into the dermis. These invaginations are termed rete-pegs, however it should be born in mind that these are in fact 3-dimensional network structures. The epidermis is commonly divided into several layers based on light microscopic findings. (Figure 9) These include the so-called granular layer and spinous layer which are derived from nuclear and organelle breakdown products and intra-cellular bridges respectively. The most important layer in terms of cytokinetic activity is that of the basal layer. It is this layer which replaces the cells lost through surface desquamation or damage. Basal cells in the human epidermis are cuboidal or columnar with their long axis perpendicular to the surface plane of the skin. The keratocytes of the basal layer include both proliferative cells (keratoblasts) and non proliferative keratocytes. It is impossible to accurately quantify the exact proportions of these cells and indeed the exact point in or above the basal layer where differentiation and maturation take place. All basal cells have a primitive keratin based cyto-skeleton, however histologically it is clear that increased keratinisation is restricted to the suprabasal layers.

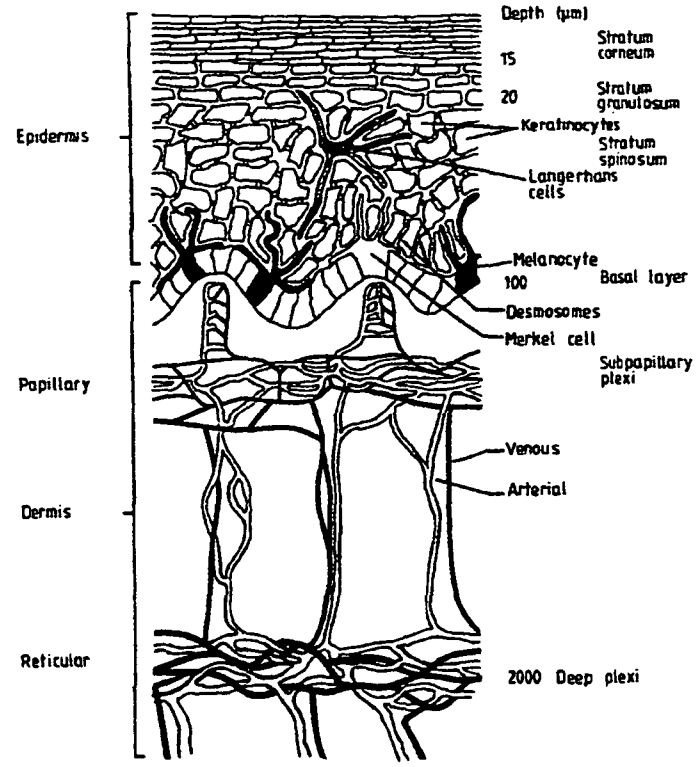


Fig 9. Diagrammatic cross section through normal skin (Feather et al. 1989)

5.1.2 **Kinetics**

In functional cell kinetic terms, a critical distinction must be made between a stem cell and a non-stem cell. We can define stem cells as those cells in an adult tissue that are ultimately responsible for all cell replacement including their own. For example, in the laboratory mouse, a marrow stem cell may undergo two hundred divisions in the life of a laboratory mouse and then be grafted at least five times into further mice. Hence from the point of view of an individual mouse, its marrow stem cells can be regarded as immortal since they have division potential which is many times greater than is required by an individual mouse life span. Because of their large division potential and their role of the as the ultimate ancestors of all other cells, the stem cells of the skin are most likely to be the cells responsible for tissue regeneration after physical, chemical or radiation damage. Because of the physical and ethical difficulties involved in sectioning human skin, most data on the distribution of stem cells has been derived from mouse experiments. These included tritiated thymidine administration and

labelled nuclear counting, percent labelled mitosis techniques and continuous labelling techniques. These results suggested that the proliferative compartment in mouse epidermis is heterogenous and contains relatively few stem cells (10%-12%). Potten (1985) has suggested that a proliferative model such as Figure 10 might describe the pre-migratory kinetics of the epidermis.

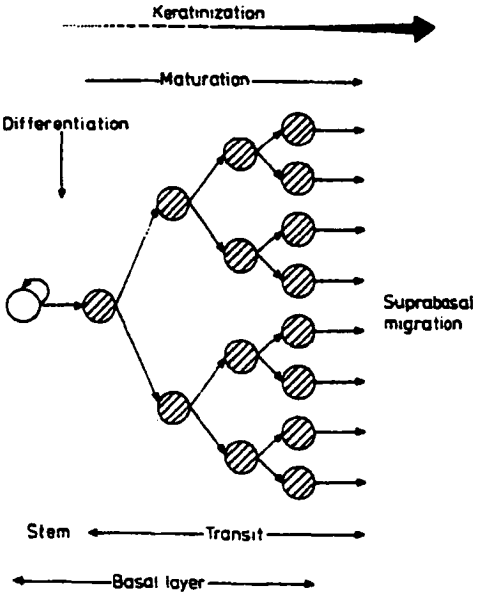


Fig 10. A diagrammatic representation of the model which it is believed most adequately explains the cell replacement process in mouse epidermis.

Cell cycle times can be measured in-vivo using a variety of techniques, however the most direct and accurate is probably the percent labelled mitosis technique. Technical difficulties have meant that comprehensive studies have been few. Table 2 indicates cycle times and S Phase durations measured for various small mammals and man. Other difficulties with these techniques, include the possibility that at least two subsets of cells with different cell cycle times may be present, and that Circadian rhythm variations may impact on both the timing and synchronicity of cell replication and therefore that artificial errors may have been introduced with the use of empirically based "convenient" skin sampling times by the investigators.

The mechanisms which are responsible for migration of cells to the basal and suprabasal layers are uncertain. It is likely that G_1 cells in the basal layer randomly migrate upward, altering basal cell

density and that this reduced density forms the stimulus for further division. The evidence for this theory comes from three sources: -

i) simple histological observation of the epidermis from the back of a mouse indicating that basal cells are not packed tightly together.
ii) minor wound healing in the mouse results in rapid immigration of basal cells which is then followed by a burst of mitotic activity.
iii) experiments in guinea pig epidermis and more notably in the mouse intestine indicate that cell migration continues even when mitosis is stopped by radiation or drugs.

Most of the relevant cell kinetic data for man has been deduced and extrapolated from other animal data and comparisons of the cell populations in the mouse and human epidermis are given in Table 3. This table assumes a three layer transmission cell compartment model.

TABLE 2

Cell cycle length and the duration of S phase as determined from percent labelled mitosis experiments							
Strain	Site	Sex	Age (weeks)	^{3}HTdR dose (μCi/mouse)	Length of S phase (h)	Length of cycle (d)	References
hr/hr	Dorsum	♂ ♀	8	10	5.5	-	Iversen et al. (1968)
		♂ ♀	8-13	~25	7.2-11.2	-	Clausen et al. (1981)
Swiss A	Ear	♂	16	25	20.2	-	Laurence and Christopers (1976)
	Sole or foot	♂	16	25	19.7	-	Laurence and Christopers (1976)
StA	Lumbar	♂	4-8	25	8.5	-	Olsson (1976)
SAS/TO	Dorsum	♂	8-9	25	11.5	4.2	Hegazy and Fowler (1973)
C57BL	Dorsum	♂	20	20 100	~16	~3.25	Gelfant in Potten (1981)
	Ear	♂	20	20 100	~11	~5.0	Gelfant in Potten (1981)
DBA-2	Dorsum	♂	7-8	25	8.5	3.1(9.1)	Potten (unpublished)
	Ear	♂	7-8	25	12.0	3.5(7.1)	Potten (unpublished)
	Tail	♂	7-8	25	12.7	3.5(7.1)	Potten (unpublished)
	Foot	♂	7-8	25	~12.5	~3.2(8+)	Potten (unpublished)
Guinea-pig	Body skin	♂	Young	5 i.d.	9.5	-	Yamaguchi and Tabachnick (1972)
Man	Body	-	-	5 i.d.	16	-	Weinstein and Frost (1969)
	Psoriatic	-	-	5 i.d.	8.5	-	
	Psoriatic	-	-	10 i.d.	7.7	-	Duffill et al. (1976)
	Psoriatic	-	-	10 i.d.	10	3.8	Goodwin et al. (1974)
Man	Psoriatic	-	-	-	-	6.8 days	Gelfant et al. (1982)
Large White Pig	Flank	♀	12-16	10	7-10	5-6	Morris and Hopewell (1986)

Table 2 Cell kinetic data for various small mammals and man. Figures in parentheses are the possible values for a second cell population with a long cycle - the stem cell compartment in some models of epidermal proliferative organisation. i.d. = intradermal

TABLE 3

	Mouse	Man
Cornified cell layers	~6	>10
Nucleated cell layers[a]	~4	> 5.5
Transit tome of a cell through the layers	8 days (range 5-11)	~24 days (range 12-48)
Transit rate (cell layers per day)	~1	>-1
Basal layer: Stem cells (St) Transit cells T_1[b] T_2 T_3	 ~10-12%[c] T_c=180h ~12% T_c=180h ~12% T_c= 90h } T_c=126hr ~25% T_c= 90h	 ? ? T_c=125(very wide ? range in ? estimates)
Post-mitotic cells(Pm)	~25% $T_{½}$=30h	?
Melanocytes	~ 3%(~400/mm^2)	0.3-2.4% of epidermal volume (700-1600mm^2)[d]
Langerhans cells	~10%(800-1400/mm^2)	1-2% of epidermal volume (400-1000/mm^2)
Proliferative fraction	0.61	?
Cell production (cells/day/basal cell)	0.13	?

Table 3 Cell populations in mouse and human epidermis.

[a] including the basal layer

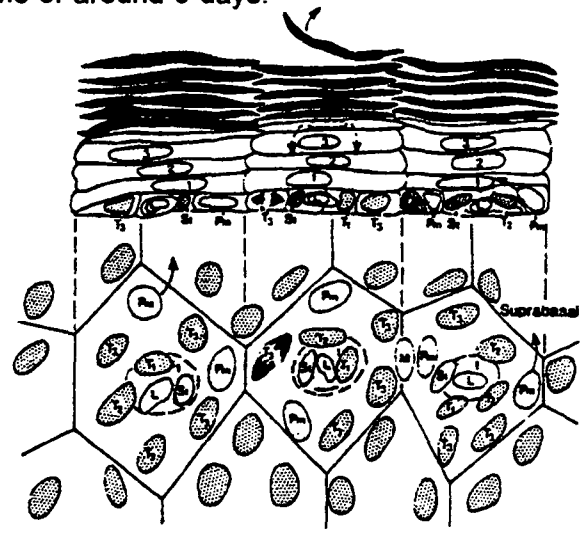
[b] T_1-T_3 are first to third amplifying transit cell divisions

[c] Percentage of all basal cells

(Potten 1985)

5.2 The "EPU" Concept

Potten has developed the concept of an epidermal proliferative unit (EPU), each of which contains approximately 10-11 cells, most of which are basal cells, although on probability there is likely to be a single Langerhans cell, Merkel cell or infiltrating lymphocyte. (Figure 11) Cell kinetic studies suggest that the more slowly cycling cells are located towards the centre of a group of basal cells. Three or four of the basal cells tend to be clustered towards the centre of the outline of the EPU with the remaining cells spaced further apart towards the periphery. In this model it is assumed that the centrally located stem cell or cells tend to have longer cell cycle times perhaps in the order of 180hrs and the surrounding proliferating cells of two or three compartments may well have shorter cell cycle times in the order of 90hrs. If one accepts this model of an EPU the destruction of stem cell would result in the loss of all the basal cells in the EPU in a time of around 9 days.



						Average phase length
Cell cycle times (h)	180	180	90	90	P½=30	126
Length of S phase (h)	2.5	8	15	15	P(av)=45	14.1
Approx % of keratocytes	14	14	14	28	28	

Stem cell divisions/lifetime = 130

Cell production/day ~ 10^7

Cell production/lifetime ~ 10^{10}

Fig 11. Diagrammatic representation of the proliferative cellular organisation of the epidermal proliferative unit (EPU) of the mouse.

5.3 Keratin

Keratin is a complex protein manufactured into a filamentous network which fills the keratinocytes. The protein network is rich in disulphide linking bonds and as a consequence it is a very tough insoluble molecule. The functional integrity of a mature keratinocyte is achieved by the heavily cross linked keratin filaments and the protein is only detected in the larger cells after they leave the proliferative compartment, that is, the basal layer of the epidermis.

5.4 Blood Vessels and Capillaries

The vessels of the dermis form rich networks throughout the integument and particularly around structures such as hair follicles. The functions of the capillary network are manifold and include thermo-regulation, inter-cellular communication, oxygen absorption, excretion, immune responsiveness and tactile sensation. These capillaries are particularly evanescent in their flow rates and dynamics. The arteries and arterioles of the skin are possessed with muscle cells in the vessel wall, however the capillaries lack any muscle or organised elastic tissue and hence consist merely of an endothelial tube with a diameter of approximately 10 micrometers. Fibroblasts and pericytes are also found in close apposition to the endothelial cells and considerable doubt revolves around whether the pericyte may be involved in a contractile response also. Upstream from the capillary networks are plentiful arteriovenous "shortcuts". At the commencement of each capillary network a final muscle cell is found, the so called pre-capillary sphincter. The activity of this muscle at the final part of the arterial tree is predominantly influenced by local chemical and hormonal changes (auto-regulation). Local mediators are released from various cells including mast cells and are capable of adjusting blood flow, vascular permeability and alter endothelial growth and structure. (Biochemistry and Physiology of the Skin, 1983 Vol 2 Chapter 34; Dermatology in General Medicine Vol 1 1987; Textbook of Dermatology 1988 Chapter 41; Effects of Ionising Radiations on Connective Tissue 1983 Part 2; Potten 1985).

6 RADIOBIOLOGY: GENERAL PRINCIPLES

6.1 Modelling Radiation Lethality

One of the key concepts in understanding the biologic expression of injury resulting from any given radiation dose, is that energy deposition from penetrating X-rays at least over a microscopic scale must be of a random nature. In early studies of the radiobiology of simple organisms such as

viruses and bacteria this principle was clearly demonstrated. Radiation lethality in such a system is described by the Poisson Law. That is, if we assume an average of 1 lethal hit per cell delivered in a random fashion then $E^{(-1)}$ (equal to 37% of cells) will experience no hit and therefore survive. An equal increment in the number of hits would produce a probability of survival $E^{(-2)}$ (.37x.37). Thus, a geometric decrement in survival may be plotted as a straight line on a semi-logarithmic coordinate system. The general expression for this process is of the following form:

$$S = E^{-DK}$$

The pivotal principle involved in this function, is that for any given equal dose increment, the same proportion of cells are killed and not the same number. S is the fraction of surviving cells of an initial population, K is a constant specific to the cell population (Gy^{-1}) and D (Gy) is the given dose. This form of cellular killing is referred to as a linear or straight line relationship.

6.1.1 **Mammalian Cell Survival Curves**

The first survival curve for mammalian cells (Hela) was obtained by Puck & Marcus 1956. Cells derived from a human Ca. cervix line were irradiated in vitro and their viability was assessed according to their ability to form visible colonies in culture medium. Subsequent mammalian cell survival assays either in-vivo or in-vitro have all tended to conform to a similar overall shape. The essential features of this curve are usually a shoulder, followed by a linear or near-linear tail out to high doses. Exact agreement on whether the first portion of the shoulder is linear and on whether the tail continues to slowly bend is still awaited. A variety of mathematical models have been applied in order to describe these observed curves (Alper 1984). All these models can be used to deduce a curve shape that is consistent with experimental data, but it has not been possible to choose amongst different models on the basis of accuracy of fit to all the experimental data. Insufficient precision exists in data of this nature and when this is combined with the very subtle quantitative differences between the predictions of models, generally speaking no clear picture emerges.

6.1.2 **The Linear-Quadratic Model**

The most widely used of the mathematical models include the multi-target, multi-hit model (Hall 1988) and more latterly the linear

quadratic model (Barendsen 1982). This model was first employed in relation to radiation damage by Kellerer & Rossi (1972) and Chadwick & Leenhouts (1973). The central tenant of the linear quadratic model is that radiation effect results from two distinct processes. In the first process, a sensitive site within the cell is irreversibly damaged in one single event. In the second process, the sensitive site is damaged by separate radiation events and if only one event occurs, then this is insufficient to kill the cell. In this situation we consider the cell to be sublethally damaged. The general expression of the linear quadratic model is as follows:

$$S = e^{-(\alpha D + \beta D^2)} \qquad \textbf{(6)}$$

where S represents the surviving fraction, D is the given dose and α and β are constants which have theoretical significance in terms of the relative contributions to each of the two processes alluded to above. This model assumes that only 2 sensitive targets may co-operate to produce cell lethality, however, it is recognised that 3 or more sensitive targets may do this and therefore cubed and higher dose terms may theoretically be possible (Schultheiss et al. 1987). Figure 12 illustrates in general diagrammatic terms the components making up the linear quadratic model.

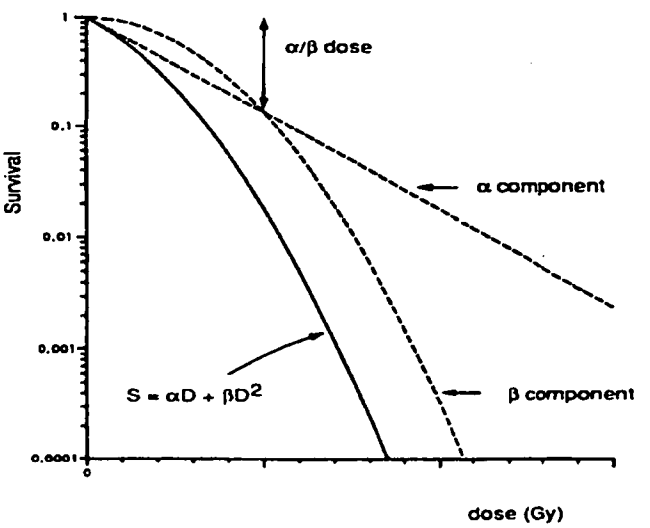


Fig 12. Linear quadratic cell survival model. The α component represents lethal lesions produced by a single track. The β component represents lethal lesions produced by the co-operation of two independent tracks. The ratio α/β is the dose at which the respective contributions of the α and the β components are equal.

Mathematically the linear quadratic model is continuously bending and therefore, has no true linear portion, which differentiates it subtly from the multi-target, multi-hit model and other repair equations.

It is theorised, that as the radiation dose increases, the density of radiation events likewise increases and the second of the two processes of radiation lethality referred to above becomes more dominant over and above the single hit process. When the two processes produce equal contributions to overall cell killing, (that is, $\alpha D = \beta D^2$), then that particular dose is numerically equal to the ratio α/β.

During the 1980's the linear quadratic model has gained general acceptance as an appropriate model for quantifying a biological response (Barendsen 1982; Moore et al. 1983; Stuben et al. 1991; Yaes 1988; Fowler 1986; LeBesque et al. 1986; Tucker 1984; Travis and Tucker 1987; Ang et al. 1987; Bentzen et al. 1989; de Boer 1988; Hendry and Moore 1985; Wong et al. 1992). The major documented deficiencies of the linear quadratic model, lie in the high dose range where it appears that most experimental observations indicate that the tail of the curve from a mammalian cell survival at high doses is truly linear rather than continuously bending as the linear quadratic model predicts. In practice, however, this only becomes modestly significant for doses in excess of 10 Gy, which is largely irrelevant for practice of clinical radiotherapy.

6.2 **The Effect of Dose Fractionation**

Early split dose in-vitro cell studies clearly demonstrated that if a dose is divided into two or more fractions, the initial observed shoulder of the curve is repeated at each fraction. This phenomenon has almost universally been interpreted as reflecting the ability of mammalian cells to repair radiation lesions between fractions. The size of the shoulder exhibited between each fraction depends essentially on the size of the fractional dose. Thus, for a very large number of small fractions cell death is predominantly by lethal damage and the cumulative survival curve follows a simple exponential (linear) function. At this level the exponential curve represents the limit to the sparing effect of any fractionation. (Travis et al. 1987; Joiner & Denekamp 1986). The size of the shoulder is reflected in a small value for the α/β ratio and therefore a higher sensitivity to fractionation and conversely a narrow shoulder associated with a large α/β ratio implies a low fractionation sensitivity.

6.3 Repair Modelling

In-vitro data from CH_3 cells (Elkind 1965) and from in-vivo experiments (Denekamp 1973; Ang et al. 1985) indicate that recovery kinetics maybe quite reasonably described by a first order expression involving time. The expression is:

$$N = N_0.e^{-\mu t} \quad (7)$$

Where N_0 is the number of sub-lesions present for interaction at the initial time (t=0), N is the remaining number of sub-lesions after any time t and μ is the time constant of the repair kinetics. (hr^{-1}). It is assumed that the repair constant μ does not relate in any way to the level of effect. In a manner analogous to isotopic decay, the half-time of repair is expressed as:

$$t_{½} = \frac{ln2}{\mu} \quad (8)$$

The methodology that is used to derive repair constants in the in-vivo and in-vitro situations are disparate. That is, in cell culture experiments, two fractions of constant size are delivered with increasing intervals and the resulting gains in survival are recorded. In tissue systems however, the relationship between cell survival and effect is unknown. Therefore, split dose experiments are carried out, keeping a constant level of effect and the parameter investigated is the additional dose necessary to compensate for repair between fractions. This concept of repair has been integrated into the linear quadratic model by Thames (1985), giving the "incomplete repair model", whose basic formula for two fractions separated by an interval of delta t is:

$$-Log(S) = 2\alpha D + 2\beta D^2 + \theta(2\beta D^2) \quad (9)$$

$$where\ \theta = e^{-\mu \Delta t}$$

6.4 Repopulation and Slow Repair

Quite apart from the effect of repair between fractions, the protraction of a given fractionated course over longer time periods, will also reduce the effectiveness of radiation, particularly on early reacting tissues. Given the rapid proliferation rate of these types of tissue systems, this time sparing effect is likely to be related to the proliferation of clonogenic cells occurring during treatment. This phenomenon has been documented by Ang et al. (1985). This phenomenon has also been demonstrated by Denekamp (1973), Morris and Hopewell (1986) in mice and pigs respectively. The exact kinetics of this phenomenon have not yet been adequately described to suggest meaningful models at this stage. Late responding tissues are much less influenced by repopulation. In the rat cervical spinal cord, repopulation is shown to be negligible within 8 weeks after the initiation of irradiation (van der Kogel 1979; White and Hornsey 1980).

In some late reacting tissues, a third time sparing effect has been described which can not be explained by repopulation. This has been attributed to "slow repair" (Field et al. 1976; Turesson and Thames 1989) The underlying mechanism of this phenomenon is not yet understood.

6.5 Application of the Linear-Quadratic Model to Organised Tissue

Radiation biology workers in the early 1980's (Barendsen 1982; Thames et al. 1983) considered that similar reasoning could be applied to gross organ end-points. For any given single target cell population within the organ we may consider that any level of clinically measurable effect E may be monotonously related to a given target cell survival S by the following formalism

$$E = -Log(S)$$

and therefore from the previous linear quadratic model

$$E = \alpha D + \beta D^2 \qquad \textbf{(10)}$$

Measurable tissue end-points in animals and humans have been found to correspond to a more limited range of target cell survivals than the in-vitro cell survival methods. Thus, unless target cell depletion exceeds something of the order of 50% then no measurable effect may often be

discerned and at the other end of the scale when surviving fractions fall below 1% then irreversible damage and or animal death may occur. Douglas & Fowler (1976), were the first workers to apply the mathematical theory of the linear quadratic model to observed erythematous reactions in mouse skin. For fractionated radiotherapy;

$$D = Nd$$

where N is the number of fractions and d is the dose per fraction. If we substitute that into the previous equation then

$$E = \alpha Nd + \beta Nd^2 \qquad \textbf{(11)}$$

therefore

$$\frac{1}{D} = \frac{\alpha}{E} + \frac{\beta d}{E} \qquad \textbf{(12)}$$

This enables a straight line plot of 1/D against d on a linear graph. α/E is the intercept and β/E is the slope of the straight line so formed. This plot is derived by determining the total dose necessary to produce a given effect (iso-effect) using various fractionation schemes (or different dose rates), plotting the data and performing a least-squares regression analysis. A disadvantage of this approach, is that all experimental data grading the tissue effect under consideration, is reduced to only one iso-effect level, thus, "wasting" considerable measurable data. A more statistically elegant approach has been suggested by Thames et al. (1986), based on a direct quantal response analysis. Other authors have employed alternative approaches, with Hendry & Moore (1985), utilising the steepness of dose incidence curves for tissue failure to derive absolute values of alpha and beta. Tucker (1984), Stuschke (1989) and de Boer (1988), have developed further indirect approaches which appear to give reasonably consistent results in terms of grouping and differentiating α/β ratios for acute and late reacting systems. Equation 12 maybe rearranged to display an organ-based "survival" curve, by plotting effect per fraction against dose per fraction. The resultant curve displays the familiar, continuously-bending linear-quadratic shape.

$$\frac{E}{N} = \alpha d + \beta d^2 \qquad \textbf{(12a)}$$

Subsequent modifications to the linear-quadratic formula have included an adjustment for possible tissue repopulation after treatment. In its simplest form, this adjustment consists of a subtracted factor which reduces the overall effectiveness of any given dose .

This is given as

$$\frac{\mu T}{\alpha}$$

where μ is given as

$$\mu = \frac{ln2}{T_{pot}}$$

where T_{pot} is the potential doubling time of the cells under consideration.

As there maybe some delay in onset of repopulation, this expression maybe further elaborated by introducing a "kick-off" time T_k. In this instance the repopulation correction factor becomes

$$\frac{\mu(T-T_k)}{\alpha T_{pot}}$$

where T is the total treatment time in days and T_k is the time in days until repopulation commences.

6.6 The Dose Rate Effect

There remains considerable debate in the world of clinical radiobiology, as to whether Low Dose Rate (LDR) radiotherapy is intrinsically associated with a therapeutic gain when compared with standard fractionated treatment. As most experience has been gleaned from brachytherapy, comparison of results with this modality to external beam or even different brachytherapy approaches with different dose rates, is fraught with difficulty. Recent evidence from laboratory studies, clinical studies and biomathematical considerations suggest that lowering of the dose rate itself may well be responsible for a therapeutic differential (Cooper et al. 1990; Dutreix 1989; Hall 1988; Hill & Bush 1973; Marin et al. 1991; Pierquin et al. 1985; Wilson 1978; Nilsson et al. 1990). Teletherapy using low dose rates in the brachytherapy range was developed at the Henri Mondor University Hospital, Creteil, France, by Bernard Pierquin in the hope of extending the perceived advantages of brachytherapy to the many clinical situations where brachytherapy is impossible. A trial comparing low dose rate external beam therapy (using a modified Cobalt teletherapy unit) with conventionally fractionated external beam teletherapy was reported on in 1987 (Pierquin et al. 1987). Highly significant differences in local recurrence rate were observed between patients treated by low dose rate irradiation (5/32, 16%) and patients treated with conventional high dose rate fractionation (20/32, 61%). This benefit apparently extended to an improved survival of patients in the low dose rate arm (44% versus 24%). Unfortunately, difficulties in interpretation arise because patients were not adequately randomised. Total doses and dose rates used in that study were arbitrarily chosen and no systematic observation of normal tissue end-points was reported.

For sparsely ionizing radiation such as gamma or x-rays, the dose rate is one of the other principal factors which determines the biological effectiveness of any given dose. This is particularly evident in the range of approximately 1 to 10 cGy per minute. As the dose rate is further reduced beyond this range, and any treatment becomes excessively protracted, repair of sub-lethal damage and repopulation occurring during treatment over come the effects of radiation induced cell kill and the "survival curve" will tend to a zero or a positive slope. Above the range of 10 cGy per minute, the dose rate effect becomes less important as exposures at this rate tend to mimic the effects of conventionally fractionated high dose rate treatments. Apart from the effects of repair and repopulation occurring during protracted exposure, it is possible that cell cycle redistribution may play a role in increasing the effectiveness of any given dose. The biological effectiveness of fractionated low dose rate radiation has been modelled principally by two authors in an incomplete

repair modelling situation (Thames et al. 1984; Dale 1985; Nilsson et al. 1990; Dale et al. 1988). These authors employ different mathematical approaches, however the predictions of each with respect to continuous low dose rate therapy and fractionated high dose rate therapy are identical (Nilsson et al. 1990). Dale defines a new parameter KR which is broadly equivalent to the dose per fraction d in fractionated high dose radiation. The quantity KR depends upon the parameter μ (the repair constant, units h^{-1}). Thames expresses the same character by small g.t. Dale's expression for the parameter K for fractionated low dose rate therapy is as follows:

$$K = \frac{2}{\mu}\left(1 - \frac{1}{n\mu t}\left(nY - SY^2\right)\right) \qquad \textbf{(13)}$$

where

$$S = \frac{nK\tilde{} - K\tilde{} - nK\tilde{}^2 z + K\tilde{}^{n+1} z^n}{(1 - K\tilde{} z)^2}$$

where

$$K\tilde{} = \exp(-\mu\Delta t)$$

where

$$Y = 1 - \exp(-\mu\tau)$$

where

$$z = 1 - Y = \exp(-\mu\tau)$$

Thames formalism is given as

$$C(n,\mu,\tau,\Delta t) = g(\mu\tau) + 2\frac{\cosh(\mu\tau) - 1}{(\mu\tau)^2} x\, h_n(\phi) \qquad \textbf{(14)}$$

where n is the number of fractions, μ is the repair rate constant, is the duration of a single fraction and Δt is the inter-fraction interval for 13 and 14. Is $\exp(-\mu(\tau+\Delta t))$ for 14. By substituting the parameter K into equation 12, we can therefore rewrite the Fe plot as

$$\frac{1}{D} = \frac{\alpha}{E} + \frac{\beta}{E} KR \qquad \textbf{(15)}$$

7 RADIOBIOLOGY OF MAMMALIAN SKIN

7.1 Radiation Erythema : Definitions and Units

By virtue of its location as the first port of call for any noxious agent emanating from the environment to man, it was the skin in which early workers with ionising radiation first noticed biological changes. The first reported example of a skin reaction may have been Lister, in 1896 or possibly the radiation erythema observed by Becquerel in 1901 after he carried he carried radium salt in his coat pocket for some days. In the years that followed, diagnostic and therapeutic radiation workers rapidly learnt from their patients and themselves, of the nature and severity of both the short and long term effects of radiation. Indeed until modern methods of dosimetry evolved and the roentgen was defined, the units of therapeutic radiation were quantified by the skin erythema dose (SED). This was defined as that quantity of X irradiation administered at 23cm focal distance at 180kv to a field of 6 x 8cm which produced a reversible skin reaction. A similar but different quantity is the MED or minimal erythema dose, which is that dose which will produce an erythema of an arbitrarily defined intensity in a particular percentage of individuals so treated. The MED is still used widely as a concept in ultraviolet erythema work, due to the extreme difficulties of ultraviolet dosimetry. Quite apart from the objections to grading what is a continuous spectrum of reaction, the SED was greatly hampered as a unit of measurement due to its dependence on the physical characteristics of the radiation exposure, skin site, texture, pigmentation and racial background. Hopewell (1990) has re-defined the terminology for describing the acute responses of the skin to radiation.

1 **Dry desquamation** (3-6 weeks for fractionated therapy): and a typical keratinisation of the skin due to the reduction in the number of clonogenic cells within the basal layer of the epidermis.

2 **Moist desquamation** (4-6 weeks fractionated therapy): the loss of the epidermis due to the sterilisation of a high proportion of clonogenic cells within the basal layer of the epidermis.

3 **Secondary ulceration** (>6 weeks fractionated therapy): secondary damage to the dermis as a consequence of dehydration/infection when moist desquamation is severe and protracted due to the reproductive sterilisation of the vast majority of clonogenic cells in the irradiated area.

4 **Dermal Necrosis** (>10 weeks fractionated therapy): necrosis of dermal tissues as a consequence of vascular insufficiency.

7.2 Stages of Erythema

i) Early Erythematous Reaction This is a well documented phenomenon which is seen within a few hours of treatment using large doses and large fields. This response has been linked with increased capillary permeability which has been assessed using protein bound vital dyes (Jolles 1972). This reaction fades quickly and is now considered to be of little importance in influencing or predicting the main erythematous reaction.

ii) Main Erythematous Reaction This second erythematous wave begins on or about the 10th day and its intensity has been found to be dose dependent and peaks on or about the 14th day. Resolution of this phase tends to be variable but usually is prolonged no further than 4 weeks. This phase of erythema has been quite clearly demonstrated to be due to a local inflammatory reaction and the process of epidermal cell death and reduction in epidermal cellularity. (Hopewell 1986). The overwhelming majority of all radiobiological studies in the mouse, pig, monkey and human have relied on various arbitrarily defined grading scales to semi-quantify observed end-points. A typical example of one of these scales relating to mouse skin was used by Douglas and Fowler (1976) (Table 4).

iii) Late Erythema, Dermal Ischaemia and Necrosis This secondary wave of erythema has been best described in pig skin after irradiation with single doses or fractionated doses involving a small number of large doses per fraction of photons or fast neutrons (Hopewell et al. 1988; Archambeau et al. 1985; Fowler et al. 1963). This secondary wave of erythema (mid-term reaction) is not seen with increasing fractionation of the dose. It is probably for this reason that this second wave of erythema is not seen in the human situation. However, at the Chernobyl nuclear accident, some victims exposed to high energy β-irradiation displayed this late erythematous phenomenon. (Barabanova and Osanov 1990). The development of the late phase erythema reaction and of dermal necrosis is preceded by the loss of endothelial cells, a reduction in capillary density and increased separation of the remaining endothelial cell nuclei (Archambeau et al. 1984). Oedema and impaired lymphatic clearance also precedes the measured reduction in dermal blood flow.

TABLE 4

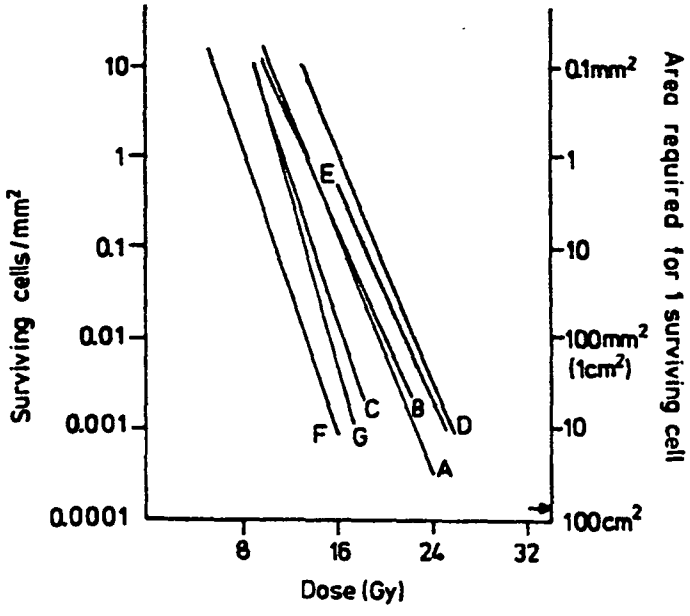
0.0	Normal
0.125	
0.25	Fifty-fifty doubtful if different from normal
0.375	
0.5	Slight hair loss and/or very slight reddening
0.625	
0.75	Definite but slight reddening ± hair loss
0.875	
1.0	Severe reddening, often with distended blood vessels or slight swelling
1.125	
1.25	Severe reddening with white scales and/or severe swelling; red "papery" skin when healing
1.375	
1.5	Moist breakdown of one small area (usually on bottom of foot first) with scaly appearance
1.625	
1.75	Moist desquamation in more than one small or one slightly larger area (tips of toes stuck together with no other breakdown when healing)
1.875	
2.0	Breakdown of larger area and/or toes stick together; possibly moist in places
2.125	
2.25	Breakdown of one-third skin area on foot
2.375	
2.5	Breakdown of one-half area of foot (usually first on bottom)
2.625	
2.75	Breakdown of about two-thirds area of foot
2.875	
3.0	Breakdown of most of the skin of foot, possibly with slight moist exudate
3.125	
3.25	Breakdown of entire skin of foot with slight moist exudate
3.375	
3.5	Breakdown of entire skin of foot with severe moist exudate; may be stuck to body fur

Table 4. Mouse dorsal skin reaction grading system (Douglas and Fowler 1976).

7.3 Epidermal Clonogenic Cell Survival

7.3.1 Mouse Skin Nodule Model

In 1967, Withers devised a technique for counting re-growth of skin nodules on the dorsal skin of mice. The technique involved sterilising several large areas with high doses of low penetrating X-rays and then exposing central shielded areas to various test doses. By counting the number of regenerating nodules and assuming that each nodule represented the survival of one basal cell the average number of cells surviving for each dose could be extrapolated using Poisson statistics. Several authors have subsequently employed Withers' technique (Figure 13) and in general have provided reasonably consistent results, with survival curves having similar D_0 values between 0.95 and 1.5 Gy. Extrapolation numbers, however, vary significantly and all the experiments suffer in terms of using different qualities and techniques of radiation, animal strains, and proliferative status of the skin (time after hair plucking). There are also considerable technical difficulties associated with this technique with respect to exudates over test areas, erratic growth of nodules and the likelihood that proliferating nodules form from hair follicle units rather than individual cells. This experimental technique does not allow for measurements of cell/colony/EPU survival at low doses and most extrapolation numbers derived from such experiments correspond to approximately 1×10^4 cells/mm^2 (a number very close to the actual basal cell density in mice). It must be assumed, with the presence of an as yet unmeasured shoulder on these curves, that the actual clonogenic number of cells/colonies is in fact considerably less than 1×10^4 cells/mm^2. This indirect evidence suggests that the epidermis contains both clonogenic and non-clonogenic **proliferating** cells.



A	Withers	1967
B	Withers	1967
C	Withers	1967
D	Emery et al.	1970
E	Denekamp et al.	1974
F	Denekamp et al.	1971
G	Leith et al.	1971

Fig 13. Survival curves for macroscopically visible colonies in mouse skin. The number of surviving cells per mm^2 of epidermal surface which produce colonies is plotted on a logarithmic scale against radiation dose. The scale on the right shows the surface area needed to provide one surviving cell. the arrow shows approximately the entire surface area of a mouse.

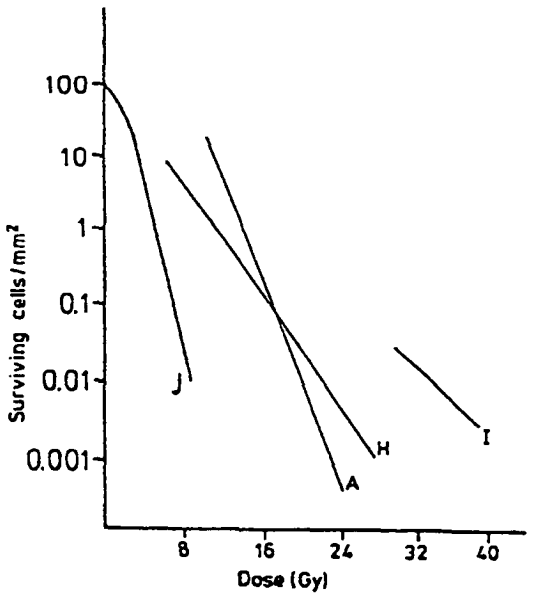
7.3.2 Histologic and Labelling Studies

Pathological changes seen in the skin after exposure to ionising irradiation include pyknosis, karyorrhexis, coagulative necrosis and apoptosis. All are seen in association with cell death. The first histological events seen prior to cell death are a combination of cytoplasmic and nuclear material, a shrinkage and rounding up of the cell and fragmentation of the nucleus and cytoplasm. Histological sectioning in animal studies has proven an extremely difficult and highly variable measure of radiation damage in terms of the time course of the various pathological end-points mentioned and there does not appear to be any clear dose relationship between the dose of ionising radiation and such changes. The absolute yield of dead and dying cells after any given dose of X-rays never appears to exceed approximately 10% of basal cell

numbers. Of course a substantial number proportion of cells may be sterilised but persist histologically unchanged in the tissue. Others still may continue to differentiate and function and take some further days or weeks to die histologically.

Sectioning pig skin some two to four weeks after radiation enables counting of viable basal cells. Various workers have defined different "runs" of healthy cells as representing a colony and attempted to plot survival curves. These results give somewhat divergent values for D_0 and are difficult to interpret in the light of other known mouse or human data. (Archambeau et al. 1979; Nyman and Turesson 1991). One of the difficulties associated with this micro-colony approach via histological sampling is to orientate healthy looking cell runs in three dimensions. For example, it maybe impossible to say whether small islands of viable cells represented sections through the middle of roughly circular colonies or sections that skimmed the edge of large or irregularly shaped colonies. Colonies associated with hair follicles or ducts of glands display similar problems.

An alternative method to gain some insight into target cell survival in the mouse model is to use auto-radiography to detect rapidly growing micro colonies. Again this technique is fraught with problems including background counts and selection of an arbitrary density of counts to qualify as a proliferating clone. Generally these experiments have produced higher D_0 values and lower extrapolation numbers than the skin nodule technique. (Figure 14) Drawing significant conclusions from any of these studies with respect to extrapolation numbers which are derived by back extrapolation to a logarithmic scale is extremely fraught. It is clear, however, from both approaches that an exponential dose dependent survival phenomenon is being measured, which must reflect an EPU either follicle associated or epidermal associated which might account for measured higher D_0 values.



A	Withers	1967
H	Al Barwari and Potten	1976
I	Hendry	1984
J	Dover and Potten	1983

Fig 14. Survival curve for human epidermal cells grown in culture (curve J) compared with the *in vivo* curves for mouse obtained by scoring microscopic (curve H) and macroscopic (curve A) colonies.

Nyman and Turesson (1991), utilised a histological basal cell density count and again encountered problems with technical factors such as fixation artefact and decisions regarding an arbitrary length of basal cells along the epidermal surface on which to count. They found that changes in basal cell density after irradiation showed a great variation from day to day but did demonstrate that depression of basal cell density and recovery, post radiation could be approximated by a linear plot of density against time. (Figure 15) They concluded that the histological method gave results consistent with macroscopic scoring of traditional gross tissue injury. They felt, in common with other authors that the labelling index was not sensitive enough to be used as a reliable end-point for quantifying acute skin effects. Morris and Hopewell (1986), performed a similar series of experiments using fractionated radiotherapy and demonstrated that the severity of damage and the rate of recovery were both dose dependent in terms of basal cell density and labelling index. They also demonstrated from their auto-radiographic studies that the proliferative response following radiotherapy was confined to the first layer of basal cells rather than

supra-basal layers. Although mitosis had been noted in the first one or two suprabasal layer pre-radiation, the percentage of mitosis in the suprabasal layers did not change post-radiation. This suggests that the suprabasal layers have limited proliferative potential when compared with the immediate basal cells. This phenomenon tends to support the concept of a hierarchical proliferative cell population (Potten 1985). These authors were also able to provide data on in-vivo measures of repopulation during a protracted radiation schedule. (6 week treatment, daily fractionation to doses ranging from 52.3 to 80 Gray). Labelling indices in the basal cells had approximately doubled by day 28, and cellular turnover time had approximately halved to 50 to 60 hours by day 28.

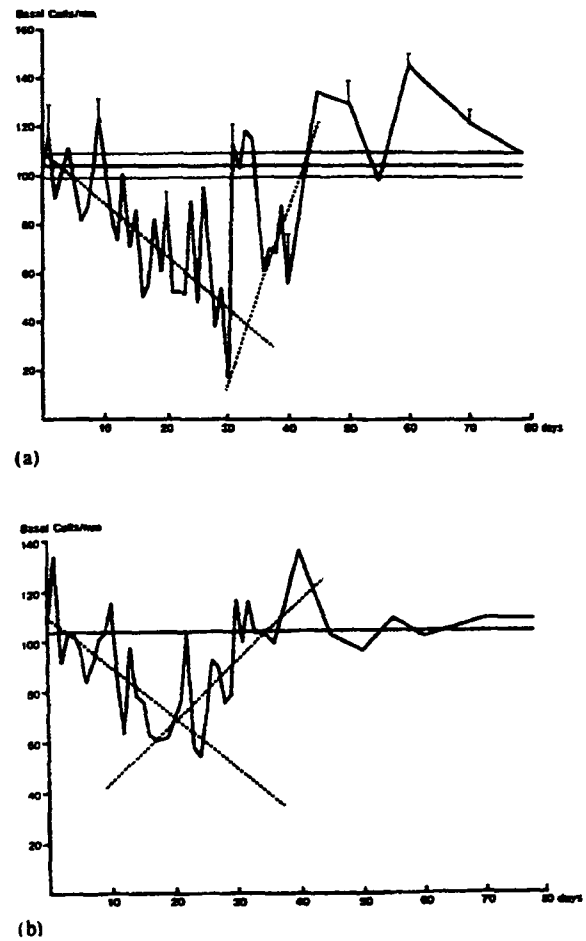


Fig15. a) Cell density variations with time after a single dose of 24 Gy with the high dose rate (1.5 Gy/min). Linear regression analysis was used for day 0-30 and 30-45 (dashed lines), with regression coefficient (r) 0.75 and 0.20 respectively. Horizontal lines indicate mean value of BCD for unirradiated skin with SD. Error bars indicate SD of each sample. b) Cell density variations with time after a single dose of 24 Gy with the low dose rate (0.02 Gy/min). Linear regression day 0-25, r=0.71, and day 25-45, r=0.67.

7.3.3 Human In-Vitro Survival Curves

Dover and Potten (1983), generated human keratocyte survival curves from circumcision specimens from young boys. Figure 14 shows their results superimposed on previous micro-colony and macro-colony data for comparison. The human cell line provided a D_0 value of 0.74 Gray which was not markedly different from the lowest value obtained in mice using the macro-colony approach. The graphical representation in figure 14 assumes that human skin has approximately 1400 colony-forming cells per mm^2.

7.3.4 Studies of Gross Skin End-Points

There have been a large number of other general radiobiological studies of predominantly mouse dorsal skin and foreleg which have used traditional gross tissue appearances to test the effects of fractionation, dose rate, dose split effects and the time factor. (Fowler et al. 1963; Denekamp 1973; Field et al. 1975; Douglas and Fowler 1976; Moulder & Fischer 1976; Turesson & Notter 1979; Denekamp et al. 1984; Joiner et al. 1986; Vegesna et al. 1988; Hamlet & Hopewell 1988; van den Aardweg et al. 1988; Vegesna et al. 1989; van den Aardweg and Hopewell 1992). These studies have all contributed to our understanding of mouse and pig skin as an acute cell renewal system which responds to radiation with cell death essentially confined to the basal cell layer and a compensatory repopulation demonstrable from split dose and top-up experiments, starting at somewhere around 9-14 days in the mouse. A similar pattern is seen in pig skin, however, Archambeau et al. (1979) demonstrated that the time of onset to epidermal regeneration was between 17 and 28 days and that the exact time of onset was dose dependent. No evidence has been found as yet to demonstrate the phenomenon of slow repair which has been suggested as a possibility in mouse lung. (Field et al. 1976) Later workers (van den Aardweg & Hopewell 1992) have performed more complete split dose studies and suggest that repair mechanisms in pig skin may be best explained with a bi-exponential equation rather than a simple mono-exponential model. They report a fast and a slow component of repair half-time values of 0.14 hours and 2.7 hours respectively.

Later workers in the 1980's have applied α/β formalism to fractionation studies in the mouse skin. Vegesna et al. (1989), derived α/β ratios from the dose-fractionation response of growing and resting mouse hair follicles, inducing proliferation in follicles by

hair plucking, pre-treatment. Their estimates for α/β ratios were 6 Gy and 3.6 Gy for growing and resting follicles respectively (dose per fraction < 7 Gy). For resting follicles, however, they noted the F_e plot was non-linear suggesting that incomplete repair was occurring between fractions or that the linear quadratic model was inappropriate to their data. Unpublished data from Withers confirms this phenomenon with higher measured α/β ratios for anagen hairs (7.7) then telogen hairs (5.5), in the mouse. This was accompanied by substantially shorter repair half-times in the anagen hair (0.63 hours versus 1.5 hours in telogen hairs). Data such as this suggests that more rapidly proliferating epidermal cells have a reduced fractionation sensitivity, more rapid repair mechanisms and shorter cell cycle times.

7.4 The "Volume" Effect

The first demonstrations of the dependence of the severity of the radiation reaction on the area or volume of tissue irradiated, was obtained by Jolles (1941) from experiments in rat skin. Various authors since that time have constructed iso-effective tables and guidelines principally derived from unquantified clinical observations, to support the existence of the volume effect (Ellis 1942; Jolles & Mitchell 1947; Patterson 1963). Hopewell & Young (1982), studied the treatment area effect in pig skin by comparing the severity of erythematous reactions and the dose required to produce dermal necrosis in field sizes of 4x4cm and 16x4cm. They found no significant relationship between area of skin irradiated and end-point. In experiments related to radiological protection, (Hopewell et al. 1986), circular areas of pig skin 5 mm to 40 mm diameter were irradiated with Strontium 90 sources. They found no change in the ED_{50} for sources of 22.5 mm and 40 mm diameter.

It seems likely that a substantial proportion of observed "volume-effects" in the clinical practice of radiotherapy relate more to local dosimetric differences influencing clinical end-points, dosage inhomogeneity increasing with increasing field size and or volume and local pathophysiological processes. For some late reacting tissues, however, there is a clearly demonstrable sigmoidal volume response curve which is currently the subject of considerable research. (Withers et al. 1988). At present, best available evidence for both human and pig skin is that there is no significant volume-effect operating for acute reactions. Clinical experience, based on studies of human skin in patients receiving radiotherapy treatment, has suggested that their may be both age, and body site, related differences in acute radio-sensitivity. For example, in some treatment centres for patients showing skin with an aged or

weathered appearance, a dose reduction of up to 10% maybe made. These observations are entirely empirical and no hard data support their use.

7.5 Radiation Effects on Vascular Tissues

7.5.1 Cellular Effects

In addition to the basal cells of the epidermis, there exists other potential target(s) in the skin and other organs of mammalian systems. Endothelial cells and associated smooth muscle cells have long been identified as responsive to radiation and have been thought for many years to be responsible for many of the functional deficits identified in various organ systems, particularly many months or years after radiation (Hopewell et al. 1986). A variety of labelling index studies have been performed on skin and other organs and uniformly demonstrate labelling indices well under 1% (Hopewell et al 1986). Comparative data is not widely available for smooth muscle cells, however, limited experiments have confirmed an even lower turnover rate for these cells (Hopewell et al. 1986). A variety of other, mainly histological sectioning experiments have shown that as expected, the morphological changes in small and medium size vessels do not appear till 2-4 months post-treatment when endothelial cell nuclear loss, micro-vascular occlusion and intimal thickening are seen. (Takahashi & Kallman 1977; Archambeau et al. 1984; Hopewell et al. 1986; Young & Hopewell 1982; Hopewell 1975).

Archambeau, Ines & Fajardo (1984) took serial biopsies from pig skin over a comprehensive time scale following irradiation and were able to derive fractional endothelial cell density and fractional change in lumen density following three separate single exposures (Figure 16a & b).

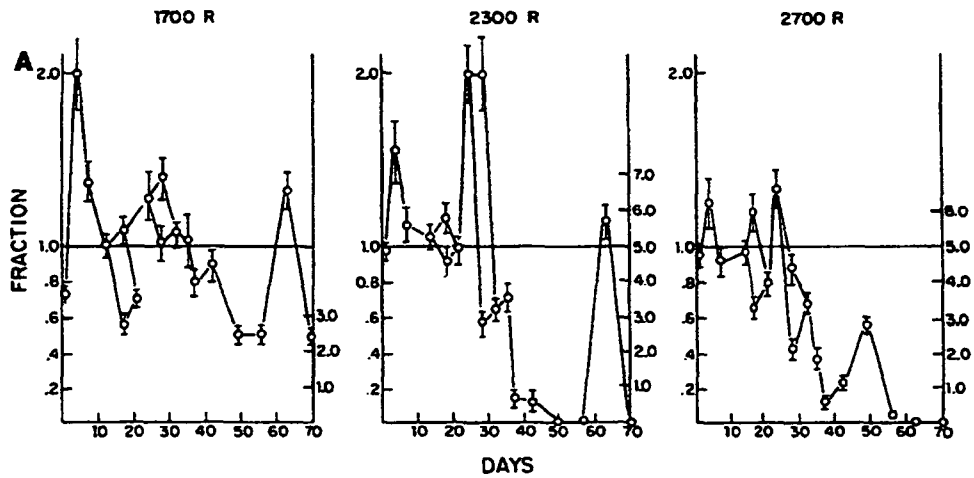


Fig 16a. Variation in time of the fractional endothelial cell density following acute single exposures of 1700, 2300, and 2700 R.

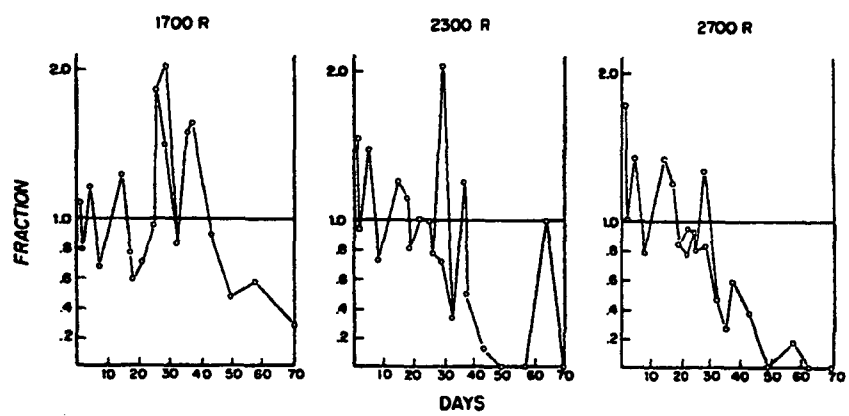


Fig 16b. Variation in time of the fractional change in lumen density following the same single exposures.

These and other studies (Hopewell 1975; Moustafa & Hopewell 1973) have suggested that the pattern of endothelial cell depletion and repopulation is similar to that reported for other cell renewal systems, apart from the elongated time scale. That is, in most other systems of an acute renewal nature there is a linear cell loss followed by an exponential repopulation. Dermal endothelium appears to manifest with a long period of no change in cell density followed by an abrupt decrease. Thus, it appears clear that the initial first wave of erythema and moist reaction following irradiation is primarily based on epidermal population loss and that further reactions or ulcerations that occur beyond approximately forty days parallel the changes seen in endothelial cells.

7.5.2 **Functional Effects**

Assessment of the functional response of the vasculature to radiation can be achieved using isotopic tracing techniques. Moustafa & Hopewell (1979), irradiated pig skin with a variety of single doses and measured clearance rates from an intradermal injection of technetium-99 ($^{99}Tc^{m}$). They identified two components of clearance in normal pig skin, a fast and slow component and were able to derive a relative clearance index for the isotope which equalled the ratio of irradiated to normal isotope clearance half-lives. This was calculated for both the fast (t_2) and slow (t_1) exponents. After a period of three weeks a decrease in the relative clearance index was observed for both components, however, by twelve weeks the reverse was true with areas of irradiated skin taking much longer to clear isotope from the site of injection than normal control skin.

This data suggests that the acute phase of a skin reaction is associated with a vasodilatation of the fine vasculature and or a change in vascular permeability. Direct histological evidence of vasodilatation in the mouse skin has been reported by Takahashi & Kallman (1977), and a wide variety of data similarly supports this concept with respect to the effects of ultraviolet irradiation. (Biochemistry and Physiology of the Skin, Goldsmith 1983). Studies in human skin to demonstrate similar functional end-points are extremely rare. Roswit et al. (1953), studied the clearance of intradermal Na^{24} and concluded that increased clearance rates were seen corresponding to the wave of clinically evident erythema. Clearance rates likewise return to normal value as erythema subsided.

Argenbright & Forbes (1982), studied the inflammatory response in the skin of pigs and mice following application of a photosensitiser and UV irradiation. They utilised a method which involved the systemic administration of ^{51}Cr-labelled red blood cells. They also used a visual erythema grading scale which consisted of comparative matching with serially layered red Kodak gelatine filters. Their results were somewhat conflicting. Mouse skin showed a nine-fold labelled red blood cell response to UVA induced inflammation but with no noticeable change in visible erythema. Conversely, pig skin was found to respond markedly with an erythematous reaction, however, an increase only by a factor of 2 in labelled skin blood content was noted. They concluded that a stimulus which produces hyperaemia need not necessarily manifest

as observable erythema and speculated that the cutaneous vascular pattern (in particular the presence or absence of capillary loops) may be a large factor in determining the potential for erythema expression in the skin. In this regard the pig skin with its well developed capillary loops similar to the human differs markedly anatomically from the hairless mouse and other rodents.

7.6 Radiobiology of Human Skin

Among the first systematic observations of acute or late skin reactions in human subjects for single dose and fractionated treatment were those made by Reisner (1933) and Coutard (1932). In 1986, Trott replotted Reisner's fractionation data on an F_e plot to derive an α/β ratio of approximately 400 R. (Figure 17)

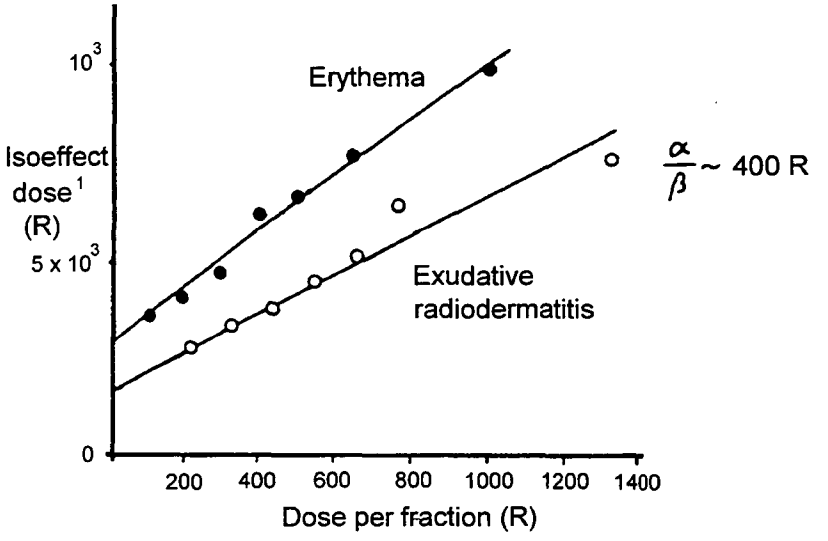


Fig 17. The relationship between inverse of total dose and dose per fraction for the same degree of pronounced erythema and exudative radiodermatitis in man. (Reisner 1933)

This value is lower than the value of 10 Gy derived by Overgaard et al. (1985) for acute radio-dermatitis after radiotherapy to the chestwall in breast cancer patients. As can be seen in Figure 17, the Reisner data is well fitted by a straight line and indeed better than many similar animal experiments. Reisner's data was collected and initially analysed some fifty years before the α/β formalism and hence an α/β ratio in the range of approximately 4 Gy is rather compelling, given that Reisner's data extended from 1-12 fractions only. Later authors, such as, Overgaard et al. (1985) and Turesson & Notter (1983) utilised fractionation schedules which were protracted over more than four weeks, when recruitment, accelerated repopulation and redistribution may become important factors over and above the repair processes operating between fractions.

A re-analysis of the Turesson data by Turesson and Thames (1989), indicated a reduced fractionation sensitivity (that is, higher α/β ratio for skin desquamation, when treatment was protracted beyond 29 days). Bentzen et al. (1987), analysed the acute erythema and subcutaneous responses in two groups of breast cancer patients to derive both α/β ratios and RBE's for the electron treatment used in irradiating the chest wall. They studied two groups of patients. The first group had post-mastectomy radiotherapy to a minimum target dose of 36.6 Gy in 12 fractions at 2 fractions per week. The second group of patients had a dose of 40.92 Gy and 22 fractions at 5 fractions per week. Using standard iso-effect plots they were able to derive an α/β ratio for erythema (at a 50% iso-effect on a standard grading scale of 1 to 3) of 10, with 95% confidence intervals of 1.8 and 22.8. This extreme range, along with the substantial dosimetric problems involved in this study (inherent with the electron beam), the case selection and physical factors involved in dosimetry and difficulty scoring uniform reactions in a surgically disturbed site, illustrate many of the difficulties associated with human studies.

The largest human data source regarding acute and late skin reactions has been derived from the work of Turesson. Since 1972 Turesson and co-workers have studied the skin reactions in the parasternal areas of almost 600 women irradiated post-mastectomy for breast cancer. This study was performed on a prospective basis, with highly standardised field location, field size, absorbed dose and radiation quality. All doses were checked with thermoluminescent dosimetry. These studies were specifically design to test the predictions of the NSD (Normal Standard Dose) and CRE (Cumulative Radiation Effect) formula in changing fractionation number, rest periods during a course of radiotherapy, and measuring the amount of repair occurring with 15 minute, 4 hour and 8 hour interfraction intervals. Patients were treated with 12 and 13 MeV electrons and 200 kV X-rays (HVL 1.2 ml of copper). The total fraction number ranged from 4-50 and overall treatment time varied from 11 days to 40 days. The fraction size ranged from 1.1 Gy/fraction (CRE V) to 7.08 Gy/fraction (DIII). Erythema was assessed both quantitatively and qualitatively. A photo-electric reflectance unit which utilised two wavelengths (578 nm. and 660 nm.) to assess absorption by oxyhaemoglobin and melanin respectively was employed. In addition weekly photographs were taken and two observers scored skin reactions on a quantitative scale.

Turesson & Thames (1989), have recently summarised all this work using direct analysis of quantal response and maximum likelihood estimation (Thames 1985). They also applied the generalised form of the Linear Quadratic model, the incomplete repair model (IR). Endpoints analysed

included erythema (reflectance measurements), desquamation (in a quantitative scale) and telangiectasia (semi-quantitative scale). They concluded that the repair capacity for human skin was similar for erythema and desquamation with α/β ratios measured between 7.5 and 11.2 Gy. They noted that as the time over which the radiotherapy was given, exceeded four weeks, repair capacity decreased and the α/β ratio increased to between 18.3 and 34.5 Gy. The authors postulate that this may be due to redistribution in the cell cycle, resulting in a net sensitisation of target cells or possibly recruitment of cells into the actively dividing phase. The authors also speculate that their data might suggest a bi-exponential repair time for acute effects with a fast component having a half time of approximately 0.3-0.4 hours and a slower repair component of 1.1-1.3 hours (for acute effects). Overall, they concluded that quantification of erythema by reflectance spectrophotometry was a reliable and sensitive method. Pigmentation proved to be a less sensitive and less radiation dependent endpoint than erythema as measured by reflectance.

Wambersie & Dutreix (1986), (Dutreix et al. 1973; Dutreix 1986), reported a total of 77 patients who were treated with bilateral supraclavicular fields using identical techniques and overall times, apart from the fractionation pattern. Each patient had on one supraclavicular area, N fractions and on the other 2N fractions. Reactions were visually scored without a grading scale, by simply specifying which supra-clavicular field had the greater peak reaction. This enabled an approximation of repair capacity to be ascertained. By assuming that full repair occurred between fractions (minimum interfraction interval 6 hours) and that repopulation was the same in both schedules they were able to derive a theoretical cell survival curve for the stem cell population under consideration, that is, the basal cells of the epidermis. This approach, like all LQ analyses also assumed that any given biological end-points which were measured or in this case compared, corresponded to a definite survival level in the relevant stem cell population and that equal end-points implied equal stem cell survival. They expressed their data using the multi-target model and derived a D_0 of approximately 4.05 Gy. They confirmed the experience of others in deriving higher values of D_r (repair capacity for late rather than early effects).

7.6.1 In-Vivo Assays of Sensitivity

Burnet et al. (1994), was able to derive 10 fibroblast strains (including 4 duplicates) from a group of patients originally treated by Turesson receiving post-mastectomy radiotherapy. In a blinded experiment they used a standard clonogenic assay to assess

intrinsic cellular radiosensitivity of fibroblasts derived from the buttocks of 19 patients treated from CRE IVb. Using standard grading of late effect they were able to demonstrate a significant correlation of cellular radiosensitivity with late effects. There was no clear association between cellular radiosenitivity and early effects. Geara et al. (1992) were unable to demonstrate any correlation between the radiosensitivity of lymphocytes and fibroblasts in the same individual (with high and low dose rate irradiation). They concluded that there was significant variation in normal cell radiosensitivity in the one individual. In another study, Begg et al. (1993) were unable to find any correlation between erythema in relation to radiotherapy post-mastectomy and fibroblast radiosensitivity *in vitro*.

7.7 **Comparison of UV and X-ray Induced Erythema**

Responses to UV irradiation by the skin are mostly concerned with erythema, carcinogenesis or pigmentation. The action of ultraviolet light on cells is different to that which occurs with ionising radiation (Painter 1973; Han and Elkind 1977). Al-Barwari and Potten (1979), irradiated male hairless mice with both x-rays and Ultraviolet radiation and used identical reaction scores for dorsal skin observations. Their reactions scales were similar to those described by others (Fowler et al. 1963). Figures 18a and 18b show the observed skin reactions for various single doses of x-rays and Ultraviolet radiation respectively. Relevant features of these curves are that both have minimum threshold doses of 1200 rads and 2 "minutes" respectively. Peak reactions showed less dependency on the dose, than on durations of reaction at any given grade. X-ray studies revealed, that irrespective of quality or dose, reactions appeared on day 5. Ultraviolet radiation, irrespective of dose, produces reactions which are visible within 1 day. Histological studies on the number of epidermal basal cells and the number of dead or dying basal cells, after doses of x-ray or UV, that produce the same level of skin reaction have shown the following.

1 Up to 20 times more cells appear dead or dying after UV over the period 1 to 5 days following treatment.

2 Within a period of three days the basal layer can be completely lost after UV doses that produce reactions of grade III. X-ray doses that produce the same reaction grade rarely show more than a small percentage of dead cells and basal cell density takes 10 to 12 days before it is significantly depleted. (Al- Barwari 1978)

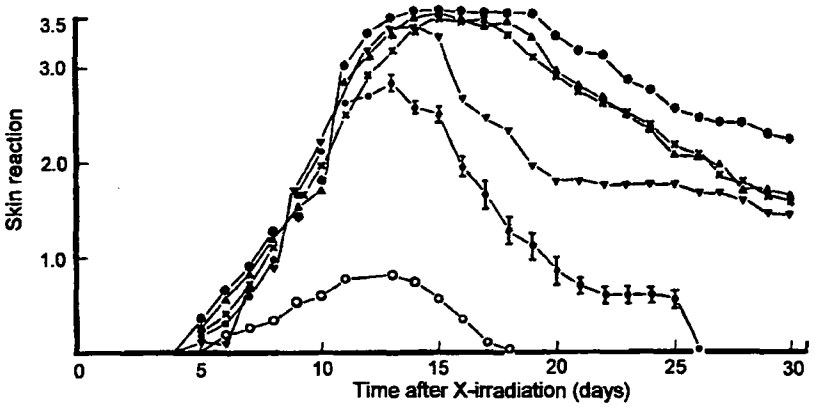


Fig 18a. Skin reactions for hairless dorsum after various doses (1200-4500 rads) of 300 kVp X-rays, plotted against time after irradiation. Each point represents the mean of five or six mice with the standard error of the mean being shown for one set of data (1600 rads). (○) 1200 rads; (●) 1600 rads; (▾) 2000 rads; (×) 2700 rads; (▲) 3500 rads; * 4000 rads. Al-Barwari and Potten (1979).

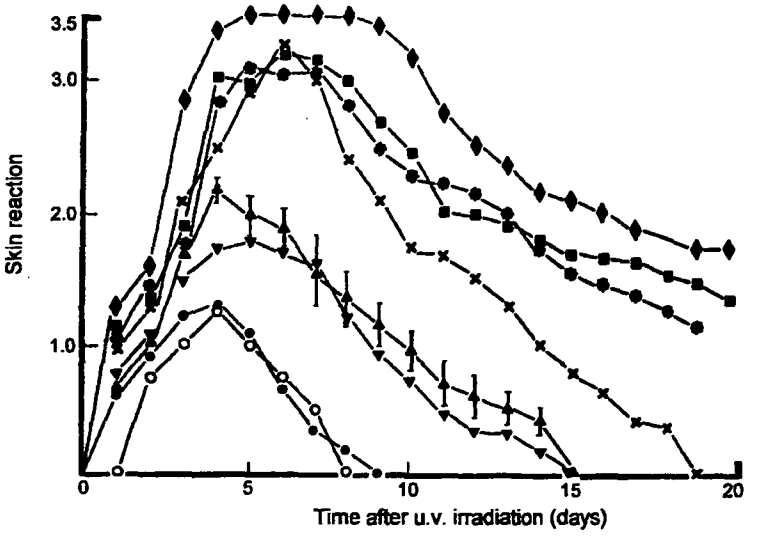


Fig 18b. Skin reaction for hairless dorsum after various doses (2-25 min exposure which is equivalent to 16-200 x 10^6 ergs/4cm^2) of u.v. (250-410nm). Each point represents the mean of five or six mice with the standard error of the mean being shown for one set of data (6 min exposures: 48 x 10^6 ergs/4cm^2). Length of exposure in min: (○) 2; (●) 3; (▾) 4; (▲) 6; (×) 8; (*) 10; (■) 15; (◆) 25.

The x-ray doses used to produce skin reactions above Grade II, must kill at least 99.9% of the clonogenic stem cells in the epidermis, (Withers 1967; Al-Barwari and Potten 1976; Potten et al. 1978). This data supports the idea that the epidermal basal layer is composed of two types of cell, a minority class, the clonogenic stem cells and a majority transitory class that are capable of proliferation but not clonogenicity and therefore are relatively unaffected by x-rays. Thus the extra cells killed by ultraviolet light in all likelihood, represent the discreet class of cells that are relatively unaffected by x-rays, that is the non-clonogenic proliferative cells.

7.7.1 **Mechanisms of Erythema**

The gross appearances and skin histological changes associated with acute radiation erythema are well documented (Radiation and Skin 1985, CS Potten; Dermatology in General Medicine Vol 1 1987 Chpt 123, Fitzpatrick et al; Biochemistry and Physiology of the Skin 1983 Vol 2 Chpt 34, A. Lowell and M.D. Goldsmith), however, less has been reported on the exact biochemical and physiological mechanisms which result in the observed capillary and arteriolar vaso-dilatation, increased vascular permeability and other associated changes. It has always been assumed that epidermal cell death produces various vaso-active mediators resulting in the above changes. In ultraviolet erythemogenesis, a large body of work has implicated a number of possible intermediate vaso-active cytokines and other growth factors which are thought to exert there action by autocrine, paracrine or endocrine modes of action. At a molecular level there is some evidence that free radical production may be involved in ultra violet light mediated erythema (Black 1987). Kelfkens et al. (1990), have employed a thermometry technique to evaluate UVA induced erythema and concluded that a vaso-active mediator released from the epidermis, diffusing into and around dermal blood vessels, represents the best model for UV induced erythema.

The study of these mediators in relation to X-rays has become known as humoral radiopathology. Prostaglandins and related compounds have received most attention in this regard (Anderson et al. 1989; Pentland et al. 1990; Imokawa and Tejima 1989; Eicosonoids in Tissue Inflammation 1993; Takahashi and Kallman 1977). The role of these compounds in modulating radiation effects has largely been deduced from experiments which measure their production following radiation and demonstrations of reduced radiation effect using drugs which inhibit their production. Michalowski has recently reviewed the literature on eicosonoid

production in relation to radiation treatment (Michalowski 1994). Eicosonoids include a number of potent natural vasoactive substances. (prostaglandins, thromboxanes and leukotrienes). These compounds are likely to be responsible for the vaso-dilatation and vaso-constriction, increased micro-vascular permeability, thrombosis and extravasation of leucocytes which are observed after radiation exposure. Many studies in small mammals and a small number of studies in man have indicated that various organs respond to radiation by rises in the level of endogenous prostaglandins and thromboxanes in the days and weeks following irradiation. Glucocorticoid steroids and NSAID's (Non-Steroidal Anti-Inflammatory Drugs) can ameliorate radiation reactions. Glucocorticoids inhibit eicosonoid synthesis primarily by interfering with phospholipase A_2 and NSAID's prevent prostaglandin /thromboxane synthesis by inhibiting cyclo-oxygnase. These studies have involved small mammal experiments using various lung, gastrotestinal and renal endpoints. Other reported possible mediators include transforming growth factor alpha (TGFα) (James et al. 1991)and bradykinins. (Pentland and Jacobs 1991)

In relation to x-ray induced erythema, Eassa and Casarett (1973) demonstrated that epsilon-amino-n-caproic acid (EACA), reduced or eliminated completely both the first and second phases of increased capillary permeability in irradiated rabbit skin. EACA is known to be a potent anti-proteolytic agent, however, its mode of action in this setting is unclear.

8 RAW DATA ANALYSIS OPTIONS

Despite the fact that this study employs a continuous quantitative variable for evaluating erythema, one still has to keep in mind that the erythema scale is likely to be non-linear in relation to effect. That is an increase in the radiation dose (and therefore the amount of damage producing an increase in x units on the erythema scale) may well not be equal for all levels of erythema effect. This point is graphically demonstrated in Figure 18a. One can see that graded erythema increments in terms of the peak erythema, are non-linearly related to dose increases. These authors, in common with many radiobiological studies have derived these average reaction scores by taking the mean score at fixed time points. This method has two disadvantages; (1): averaging is done on a non-linear scale, which is statistically incorrect and (2): reactions for individual animals or patients may peak on different days and the shape of the resultant reaction curve is modified, that is the peak will reduced, resulting in an under-estimation of the maximum reaction.

These disadvantages can partly be overcome by a second method of averaging. That is, calculating the average time at which a certain damage level is observed. In this way averaging is done on a linear scale (time) and the average curves always indicate the maximum level of reaction predicted. The gain in accuracy with this second technique is difficult to predict. In practice in radiobiological experimental practice, both these methods are employed and generally speaking give consistent results. (Vanuytsel et al. 1986).

Figure 19 shows other parameters which may have theoretical usefulness in describing reaction curves. Temporal measures such as latency for the peak and time to reach any given reaction grade tend to be generally insensitive as most acute animal and human responses are consistent and relatively independent of dose and dose rate. Given the non-linearity of association between peak and net radiation effect, the two most likely parameters useful in the human situation, where reaction curves show considerable heterogeneity, are likely to be duration of reactions at given levels and areas under the peak above a given level. The latter takes into account both the peak values and duration by virtue of its integrative nature. Table 5 summarises these analytic options. Both the duration and area under curve ("AUC") methods will be more properly treated by non parametric statistical methods rather than taking the mean values of these quantities for any given group of patients. It would seem, therefore, reasonable to rank these quantities and derive median or interpolated median values for iso-effect analysis.

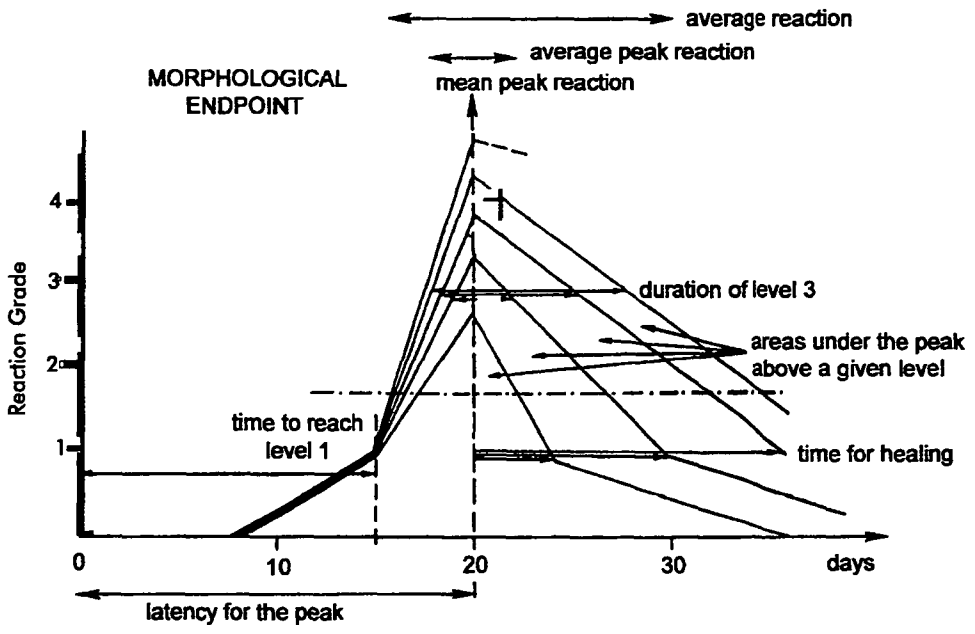
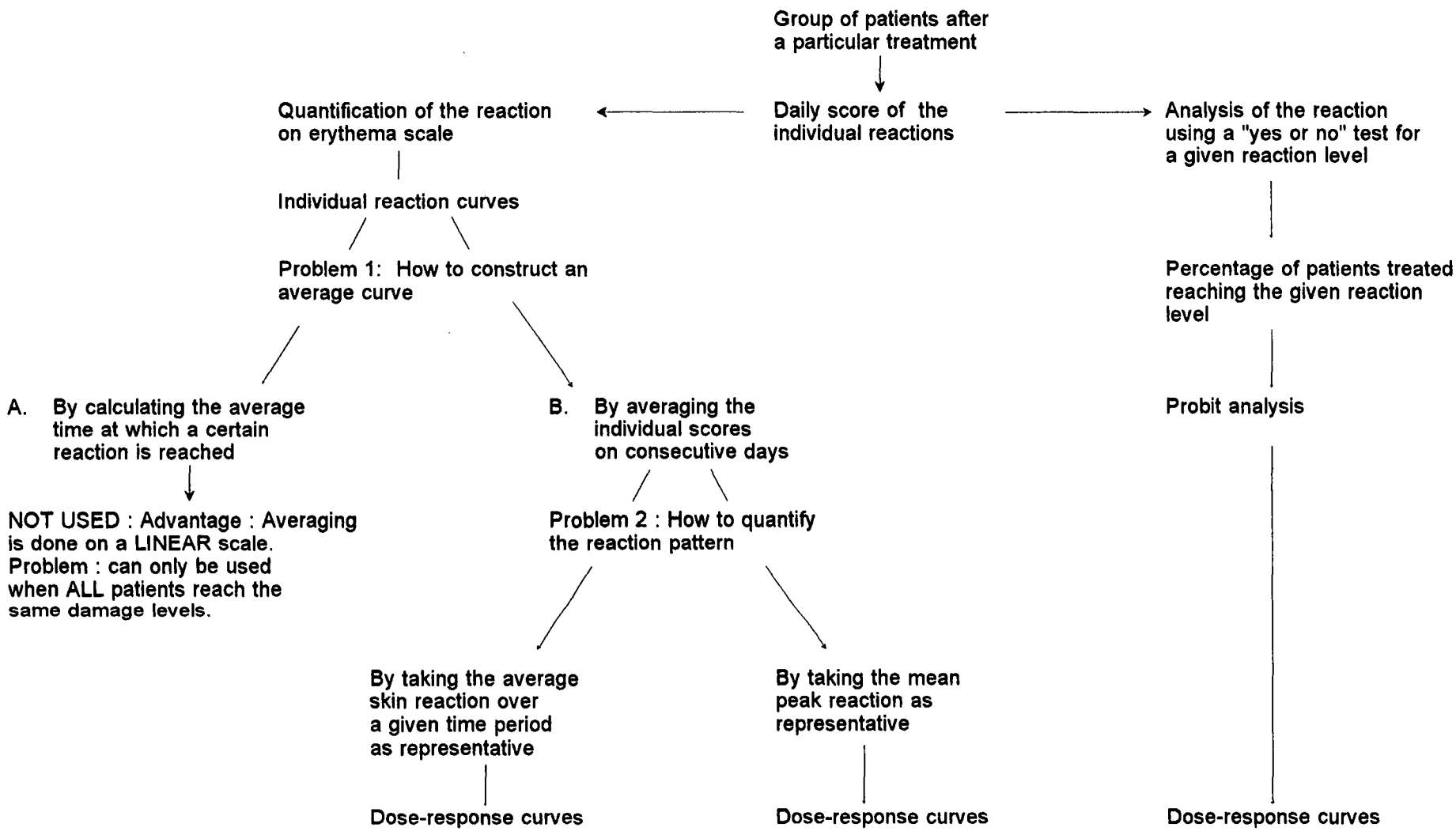


Fig 19. Schematic representation of typical acute reaction displaying the options of descriptive quantitative parameters for individual or averaged reaction curves.

When the ordinate scale is a quantitative measure such as derived from reflectance values, the investigator has the choice of utilising normalised values or an absolute change in erythema units. The normalised approach has the advantage of mathematical simplicity (with each patient beginning at a starting value of unity) and has many other physiological precendents. (eg pulmonary function tests) Because this method relies on a proportional change, it does however have the disadvantage of relatively accentuating the response at the low end of the erythema range versus the high end. By using an absolute change in erythema (taking the starting value as baseline) a more standardised measure may be obtained, but may be inappropriate for an instrument with nonlinear response.

TABLE 5


Group of patients after a particular treatment
Daily score of the individual reactions
Quantification of the reaction on erythema scale
Individual reaction curves
Problem 1: How to construct an average curve
A. By calculating the average time at which a certain reaction is reached
NOT USED : Advantage : Averaging is done on a LINEAR scale. Problem : can only be used when ALL patients reach the same damage levels.
B. By averaging the individual scores on consecutive days
Problem 2 : How to quantify the reaction pattern
By taking the average skin reaction over a given time period as representative
Dose-response curves
By taking the mean peak reaction as representative
Dose-response curves
Analysis of the reaction using a "yes or no" test for a given reaction level
Percentage of patients treated reaching the given reaction level
Probit analysis
Dose-response curves

9 RATIONALE, AIMS AND OVERVIEW OF STUDY

9.1 Rationale

The initial stimulus for this project arose from the observation that there existed no systematic quantitative observations on the effects of fractionated low dose rate radiotherapy in human skin. This situation was paralleled for hypofractionated high dose rate treatment where only the work of Turesson had been reported in the post war period. With increasing application of the linear quadratic model to experimental data since 1980, derivation of α/β ratios and t½ values for acute effects in human skin over a variety of anatomical sites would clearly be useful to augment animal data. For the safe and rationale design of new radiotherapeutic protocols (e.g. hyperfractionated or accelerated radiotherapy), accurate measures of dose rate effects, fractionation effects and repair values are required from clinical radiobiological studies such as this one. Finally, with the development of modern reflectance techniques, we had an opportunity to collect quantitative rather than qualitative measures of human erythema.

9.2 Aims

- To qualify and quantify cutaneous erythema in response to Fractionated Low Dose Rate (FLDR) and Fractionated High Dose Rate external beam radiotherapy. (FHDR)

- To test and validate Reflectance Spectrophotometry as a suitable tool for quantifying Radiation Erythema.

- To relate quantified versus qualified observations of cutaneous erythema for FLDR.

- To test the iso-effective predictions of the incomplete repair model for FLDR and FHDR.

- To derive α/β ratios and repair half-time data for human skin from FLDR and FHDR.

- To examine the influence of various other physiological and historical co-variants in radiation effects with FLDR and FHDR.

9.3 **Overview of Study Design**

This study was performed in the setting of a small to medium sized radiotherapy department, in a provincial city. The study patients were drawn from patients presenting for palliative treatment of various malignancies and patients treated curatively for carcinoma of the prostate (vide infra).

1 Patients treated as part of a clinical radiobiological research project; Low Dose Rate Teletherapy (Cooper et al. 1990; Hamilton et al. 1993). These patients were further sub-divided into two treatment groups: (10 fractions)

   A Head and Neck primary sites; treated with parallel opposed Cs_{137} portals at dose rates of 0.8, 1.8 and 3 Gy/hr.
   B Skin patients (various sites); treated with direct Cs_{137} fields at dose rates of 3, 4.8 and 8 Gy/hr.

2 The second portion of the study evolved as an extension of the Low Dose Rate Teletherapy protocol. The study population in this instance, consisted of patients treated with conventional palliative regimes (5,10,12,20 fractions at 240 Gy/hr, 6 MV X-rays) and total skin doses in the range of 4 to 52 Gy. (various sites)

3 Patients undergoing conventional external beam radiotherapy (32 fractions at 240 Gy/hr, 64 Gy, 6 MV X-rays) for localised carcinoma of the prostate were enrolled on a study assessing the response of hair follicle cortical cells to X-rays. (Potten et al. 1994) (supra-pubic only)

Both study populations 1 and 2 consisted of patients with incurable and almost invariably metastatic malignancy, having symptoms justifying palliative radiation treatment. The central theme behind utilising both the study populations, was that of deriving quantitative endpoint data (using reflectance measurements) in a setting where a range of total doses, dose rates and fraction numbers could be employed.

CHAPTER II MATERIALS AND METHODS

1 REFLECTANCE TECHNIQUE

1.1 Construction of Reflectance Unit

The portable reflectance unit used in this study is a robust integrated unit which employs 240 volt mains supply and consists of two major separate components.

1 The electronic drive and signal processing unit and

2 The skin reflectance measuring head.

1.1.1 Electronic drive and signal processing unit

Wavelength selection is via a circular variable interference filter whose rotation is driven by a computer controlled stepper motor. The spectral range from 400 to 700 mm may be scanned in 2.8 s. The light source was a 6 V, 20 W quartz halogen lamp and optical linkage between the source, measuring head and spectrophotometer is provided by the fibre optics. Incoming reflectance signals generated in the measurement head are detected by a silicon photo-diode detector, amplified and transmitted through logarithmic amplifiers (ICL 8048 CCPE). Pigment indices are calculated by simple arithmetical treatment of the incoming reflectance voltages. Measurements on a standard white surface (smooth magnesium oxide powder) are made prior to each series of experimental measurements. The readings for haemoglobin and melanin indices thus obtained are then subtracted from subsequent experimental measurements, since the definition of a white surface is that the two indices should be zero for magnesium oxide.

1.1.2 Skin reflectance measuring head

The measurement head consisted of a hollow black plastic hemisphere 2.5 cm in diameter mounted inside a metal cylinder for ease of handling and exclusion of ambient light. The illuminating optical fibre was mounted in the hemisphere at right angles to the specimen surface while the fibre collecting the reflected light was mounted at 45° to the specimen surface. A second optical channel carried light directly from the light source to the interference filter to provide a reference signal. Light intensity was measured by means of two photodiodes. The ratio of the amplified output signals was digitised and transferred to microcomputer for conversion to LIR values, storage and data manipulation.

1.2 Measurement of Haemoglobin Index

Reflectance readings were taken pre-treatment and at twice-weekly intervals during and after the course of radiotherapy, until the acute erythematous reaction returned to base line. This generally took some two to four weeks following the completion of radiotherapy. Patients were measured in a recumbent position, in an air-conditioned environment, with a constant ambient temperature of 22°C. The patient was positioned in a comfortable consistent manner and given five minutes in their recumbent position prior to measurements being taken. A suitable measurement site was selected, usually closely corresponding to field centre, over an area of flat, hairless skin. The measurement site chosen, was if possible, that displaying the least amount of previous solar damage (elastosis, hypo and hyper-pigmentation and actinic change). The chosen site was referenced using a small non-permanent skin mark which was consistently aligned on subsequent measurements with a reference mark on the external surface of the measurement probe. The probe was rested gently on the skin surface and haemoglobin and oxygenation readings each taken five times in succession. This process required approximately five to eight minutes. The mean of each of these five values was then taken to represent the reflectance reading for that day.

As has been demonstrated previously, the degree of erythema in the skin is directly proportional to the haemoglobin index and therefore we define a quantity:

$$EI = \frac{E_t}{E_0} \qquad (16)$$

where EI equals the erythema index at time t. E_0 is the erythema reading pre-treatment and E_t is the erythema reading at time t. An entirely analogous procedure may be used to calculate the oxygenation index OI.

An identical procedure was followed to measure control or background erythema and oxygenation readings outside the field. That is, a suitable point at least three centimetres beyond the field and if possible at the same vertical level on the patient as the test site was selected and pre-treatment and twice weekly readings were performed at the same time as test values were taken. (Figure 20).

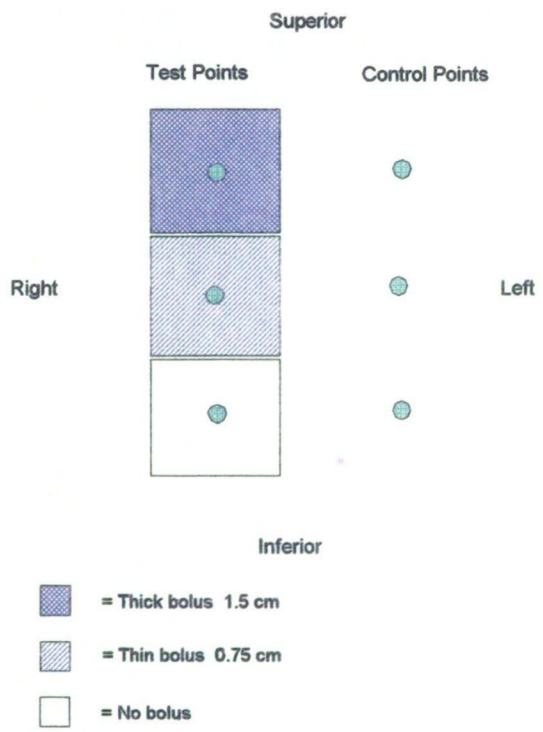


Fig 20 Schematic representation of the field sub-division (total field area greater than 175 cm^2) into differentially bolused areas along with test and control points for reflectance readings. NB. FLDR study did not include the use of any bolus as illustrated here. Location of test and control reflectance points is however entirely analogous.

1.3 Calibration of Reflectance Unit

The reflectance unit required a twenty minute daily warm-up and prior to each set of readings was recalibrated against a matt black and pure white ceramic tile. Two other additional constancy checks were performed.

1 A flesh coloured pink ceramic tile was used for daily constancy checks over a two to three week period every several months to allow checking of the constancy of haemoglobin and oxy-haemoglobin reflectance values.
2 a specific artificial absorbence filter (Wratten filter) was used on a once weekly basis, to check for constancy of haemoglobin and oxygenation indices.

Over a forty five day time period the pink ceramic tile recorded a mean haemoglobin index of 10.7 ± 0.59 (one standard deviation). Corresponding oxygenation values were 59.97 ± 21.75. The Wratten filter showed similar constancy with haemoglobin values of 53.33 ± 2.23 and oxygenation values of 52.7 ± 45.84.

1.4 Patient Consent

Following determination by medical staff that patients were suitable for either of the studies under consideration, (FLDR and FHDR), the patients were requested as to whether they would consent to their entry on either of these programs. Treatment protocols were explained in considerable detail, along with an accompanying colour photographic package to illustrate the logistics of the study. Patient consent forms for each of these studies are included in appendix 1. These studies were approved by the Regional Ethics Committee.

2 FRACTIONATED LOW DOSE RATE STUDY (FLDR)

2.1 Study Background

The Department of Radiation Oncology at the Newcastle Mater Misericordiae Hospital embarked on an experimental fractionated low dose rate (FLDR) teletherapy project in 1989. This project was designed with the aim of accurately assessing acute normal tissue end-points (mucosal and skin response) in an effort to provide a systematic iso-effect data for a range of total doses and dose rates. A modified Caesium-137 teletherapy unit was utilised for this project which ceased in 1994 with the accrual of one hundred and forty one patients. This thesis reports on reflectance data and clinical observations from skin reactions in one hundred and nine patients treated in the FLDR project. (reflectance unit arrived 1990).

2.2 Study Population

Consenting patients for the FLDR project were drawn from two categories.

2.2.1 Locally Advanced Head and Neck Cancer

These patients were assessed at a combined multi-disciplinary Head and Neck Cancer clinic and felt to have incurable head and neck cancer. If these patients were otherwise suitable for conventional palliative external beam radiotherapy, they were offered low dose rate therapy on the FLDR project. Patient selection criteria are given in Table 6a.

TABLE 6a
Patient Selection Criteria

Head and Neck Cancer	
(1)	Patients with squamous carcinoma of the head and neck that fulfil the RTOG modification of the AJC stage grouping criteria in the categories IVB, C and D and V, excepting oral cavity tumours and laryngeal tumours that may be amenable to surgery.
(2)	Patients under 80 years of age with an anticipated survival of more than 8 weeks.
(3)	Patients who do not have intercurrent illness such as dementia, severe epilepsy, incontinence, severe musculo-skeletal disorders, that are likely to make treatment lasting several hours each day difficult.
(4)	Patients who do not have severe dental caries requiring major dental work prior to treatment.
(5)	No prior treatment (radiation or chemotherapy).
(6)	Written informed consent to treatment on protocol.

Table 6a Criteria for study inclusion (FLDR)

2.2.2 Bony and Soft Tissue Metastases

The second group of patients enrolled on the FLDR project were patients with a variety of metastatic solid tumours, who would otherwise be suitable for conventional external beam palliative therapy. Selection criteria are given in Table 6b.

2.3 Irradiation Methods

2.3.1 Equipment and Technique

The Caesium-137 teletherapy unit employed in this study normally operates at treatment distances of between 20-30 cm SSD and delivers incident dose rates of greater than 1 Gy/min. Because the 50% depth iso-dose line is less than 5.5cm deep at these SSD's the use of the use of the machine is confined to the palliative treatment of relatively superficial primary and secondary tumours. Modification of this unit for low dose rate use involved the construction of a set of collimation cones using low melting point alloy. These cones improved beam flatness, beam coverage and minimised penumbra at extended SSD (Figure 21 and 22).

TABLE 6b

Patient Selection Criteria

Bony and Soft Tissue Metastases	
(1)	Proven symptomatic metastatic cancer involving sites that can be encompassed satisfactorily by modified caesium beam at 60 or 80 cm SSD.
(2)	A disease site that has not been previously irradiated, is not situated in a pressure area, and in which there is no evidence of skin or subcutaneous involvement by tumour.
(3)	The patient has not received Adriamycin, Actinomycin D, or Bleomycin containing regimes, or is being concurrently treated with cytotoxic chemotherapy or other agents that may modify radiation response.
(4)	Ability to lie still for a period of up to 1 hour.
(5)	Written informed consent for treatment on protocol.

Table 6b Criteria for study inclusion (FLDR)

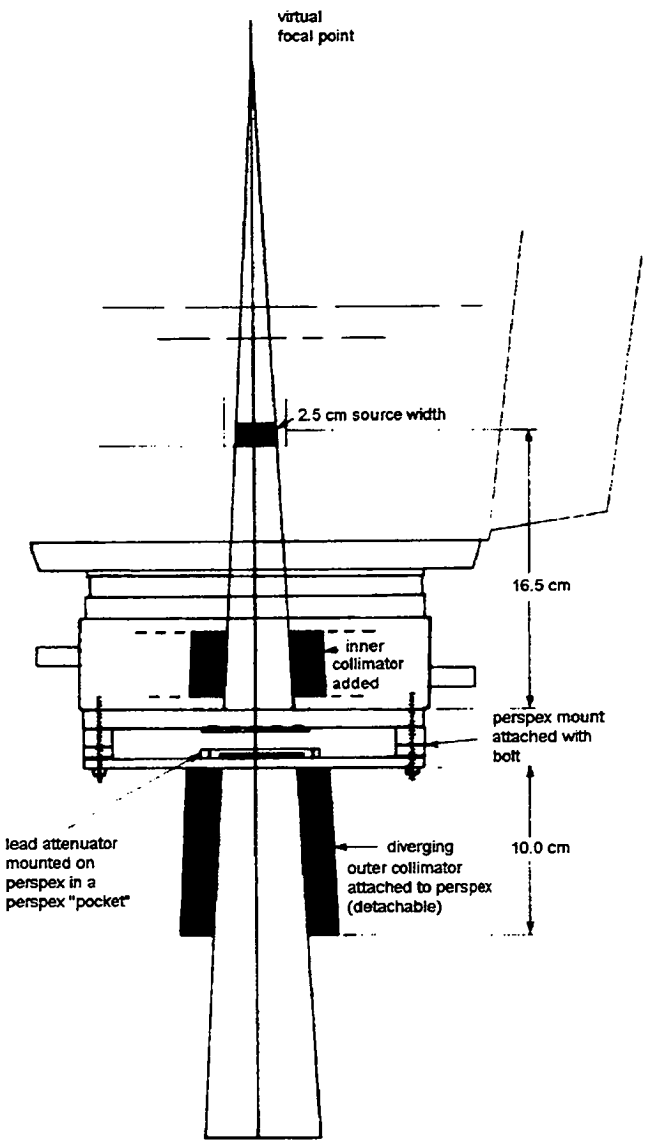


Fig 21 The Caesium teletherapy unit "head" illustrating the design and positioning of the "inner" and "outer" diverging low melting point alloy collimators in relation to the caesium source.

A series of beam flattening and attenuating filters were used to achieve instantaneous dose rates of between 0.5 and 5.0 Gy/hr at 7cm depth in tissue. This corresponded approximately to the 65% isodose, depending on field size. Patients were accepted for treatment provided that target volume heterogeneity of total absorbed dose did not exceed ± 5% using a parallel opposed field setup. A typical beam profile is shown in figure 22 and its depth dose characteristics given in figure 23.

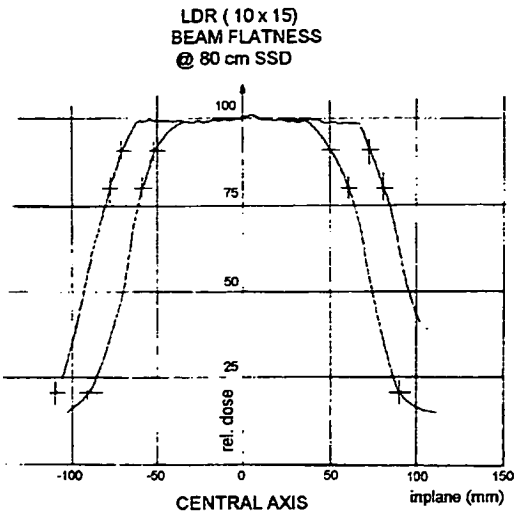


Fig 22 Beam profile for a 10 x 15 cm cone showing the flatness of the beam and its penumbra at 80 cm SSD.

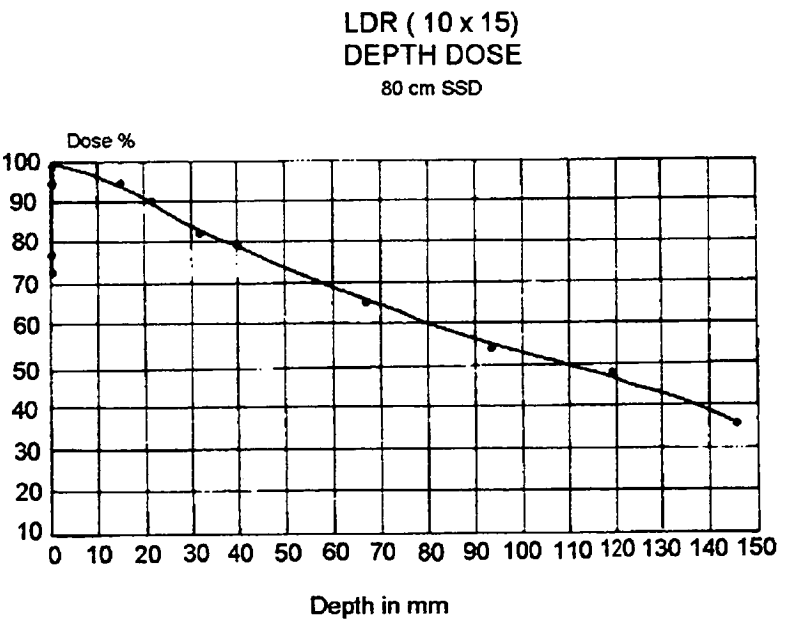


Fig 23 Depth dose characteristics of a 10 x 15 cm field at 80 cm SSD. Peak dose is estimated to be 0.5 - 0.7 mm below the surface.

Two irradiation setups were employed.

1) For Head and Neck patients, right and left parallel opposed fields.

2) For bony and soft tissue metastatic disease, a single appositional field.

Treatment times varied between 40 minutes and 5 hours per day and careful consideration was given to patient comfort and immobilisation during treatment. Patients treated for locally advanced head and neck cancer were positioned reclining on a modified dental couch with hourly rest breaks allowed. Immobilisation was achieved using a customised polystyrene head rest and velcro strapping. Patients treated with single fields had 3mm of tissue equivalent bolus placed over the entire field to eliminate the small build-up effect seen with a Caesium 137 beam. Optical proximity switches were used to confirm patient position and switch off treatment if the patient strayed from the prescribed field settings. A TV and VCR unit were provided for entertainment during treatment. The treatment itself was supervised by a closed-circuit TV. Full technical details are given in appendix 2.

2.3.2 **Doses and Dose Rates**

The range of total doses and dose rates chosen in this study were selected as a compromise between practical logistic difficulties and allowing the data envelope to encompass as wide a range of the potential influence of the dose rate effect as possible. Figure 24, conceptually demonstrates the spread of the chosen doses and dose rates which correspond to predicted iso-effect lines (Dale et al. 1988). A central composite design was chosen with the central point being represented by 38 Gy at 1.8 Gy/hr. Head and neck patients were treated at dose rates of 0.8, 1.8 and 3 Gy/hr and soft tissue and bony metastatic patients treated at dose rates of 3, 4.8 and 8 Gy/hr. Of the 141 patients accrued for the FLDR study, 109 formed the basis of this report, having had reflectance studies performed. This includes 33 patients from the head and neck group and 76 from the skin group (figure 25).

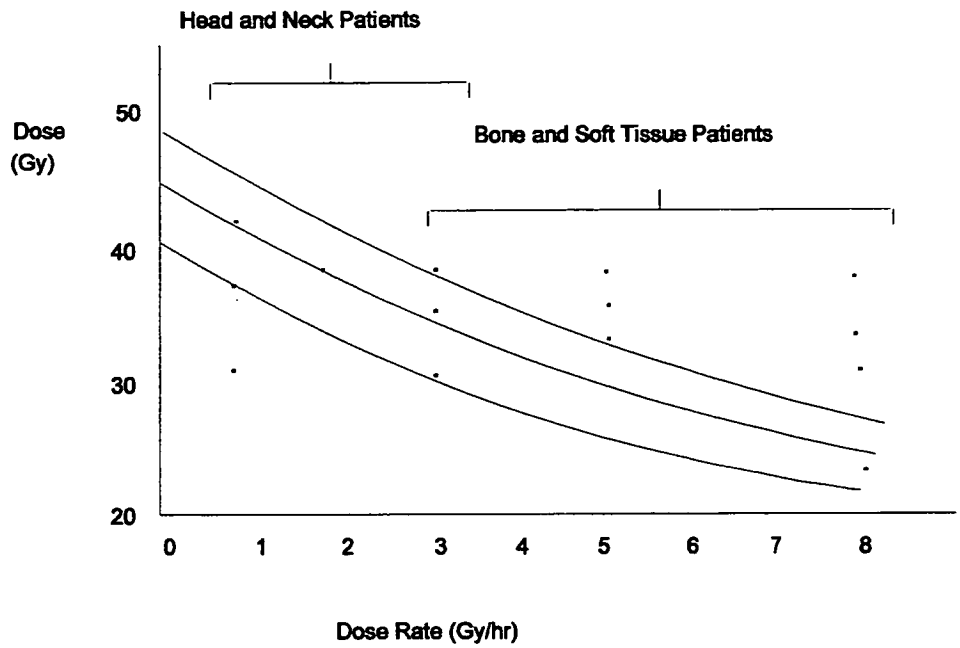


Fig 24 Dose and dose rate points chosen for FLDR study superimposed on FLDR iso-effect predictions. (Dale et al. 1988). α/β=10 T½=0.5 hours

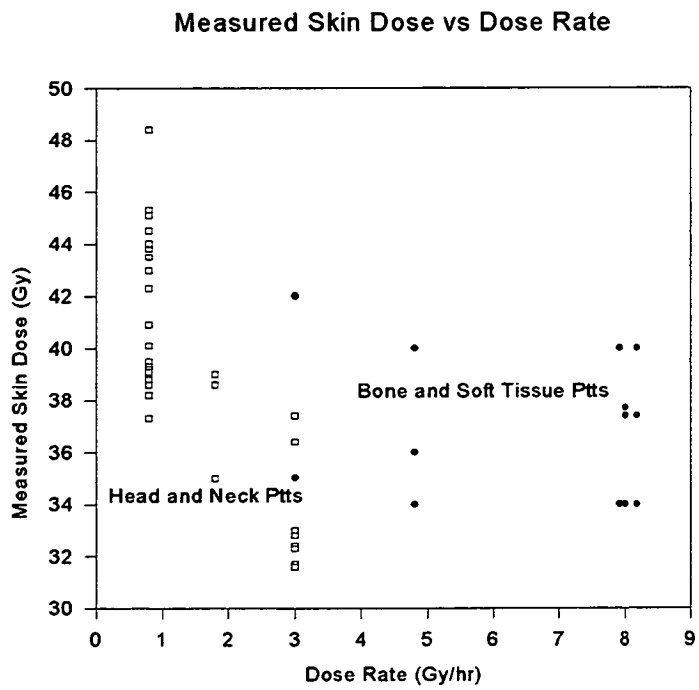


Fig 25 Matrix of measured skin dose and dose rates for the subset of patients (n=109) from the FLDR study, forming the basis for reflectance observations in this study.

2.4 Erythema Scoring

Patients on treatment were evaluated twice weekly by two independent observers who scored both skin and mucosal reactions according to a modified EORTC/RTOG scale (Table 7).

TABLE 7

EORTC/RTOG Toxicity Criteria

SKIN	
0	No change over base line
1A*	Follicular, faint erythema / epilation / dry desquamation / decreased sweating
1B*	As above but confluent bright pink erythema
2	Tender or dusky erythema, patchy moist desquamation / moderate oedema
3	Confluent, moist desquamation other than skin folds, pitting oedema
4	Ulceration, haemorrhage, necrosis

* This distinction is a local one and therefore represents a modification.

Table 7 Skin and mucosal scoring criteria for FLDR project.

3 FRACTIONATED HIGH DOSE RATE STUDY (FHDR)

3.1 Study Population

Consenting patients who were undergoing a course of palliative radiotherapy for metastatic incurable malignancy, were entered onto this study, having one or more of the following fractionation schemes.

20 Gy / 5 fx
30 Gy / 10 fx
36 Gy / 12 fx
40 Gy / 20 fx

Patient age was restricted to the range 18 to 80. The patients performance status was required to be ECOG 2 or greater and no previous irradiation to the area concerned was allowed. The patient was required to have had no previous history of significant dermatological illnesses or have had recent known photosensitising drug intake.

3.2 Irradiation Technique and Doses

Patients in the FHDR study were irradiated exclusively with 6 MV photons and suitable sites were confined to the truncal portion of the body between larynx and groin. Field configurations included single fields, parallel opposed pair and 4 field techniques. In order to generate a greater number of dose points, patients with field sizes greater than 100 cm^2, had their fields arbitrarily divided up into two sections. The first of these sections was treated through a layer of full wax bolus (for 6 MV photons, 1.5 cm). The second of the sub-divisions of the treatment field was treated with no bolus. This resulted in approximately a 30 to 40% dose differential to skin in the two areas concerned. For field sizes greater than 175 cm^2, the treated field was artificially divided up into three sections in an analogous manner with, on this occasion, the third section being treated daily through a layer of thin wax bolus (0.75 cm). Reflectance readings were done in the centre of each of these arbitrary sub-divisions of the field. (Figure 20)

3.3 Other Recorded Variables

In addition to the physical quantities and parameters already described, FHDR patients had several other variables recorded and transcribed to the Medlog data base for later analysis. These included, medications, natural hair colour in early life, natural tanning pattern, historical UV score, prior UV exposure on face and prior UV exposure in treated area. The later three of these parameters were graded on a simple semi-quantitative scale, using published criteria (Cameron et al. 1988). A comprehensive list of recorded variables is given in appendix 3.

3.4 Hair Cortical Project

Patients undergoing conventional external beam radiotherapy for localised carcinoma of the prostate (T1-T3,N0) (n=49), had reflectance readings taken (vide supra) (without bolus) as part of a study to assess the response of human hair to X-rays. Full details of the technique are given in Potten et al. (1994). In brief; approximately 100 groin hairs are removed pre-treatment and again after a given dose (in this case 4,8,16 or 32 fractions). Hair cortical cell density is measured using confocal microscopy and the fractional reduction represents a measure of follicle cell kill. Patients were treated using 6 MV photons with a 4-field box set-up to a target absorbed dose of 64Gy in 32 fractions. Care was taken to separate the test and control points from the hair sampling point . Field sizes were CT- planned to cover the prostate gland plus a margin only.

4 IRRADIATION DOSIMETRY

4.1 Introduction

The most accurate in-vivo skin dosimetric method available in radiation physics is thermoluminescence dosimetry (TLD). This method of dosimetry was employed in both the FLDR and FHDR studies and is particularly applicable due to its ability to estimate dose at the basal cell layer in the epidermis. The International Commission on Radiation Units and Measurements (ICRU report 39) recommends 0.07 mm as the most appropriate depth of measurement for the individual dose equivalent. Direct measurement of this skin dose is preferable over calculation due to the many parameters which influence the surface dose. This includes factors such as electron contamination of the original beam, field size, shadow trays, beam blocks, patient geometry and obliquity of the beam. Carbon-loaded black TLD's (Francis et al. 1989; Thomas and Palmer 1989) are particularly designed for surface dose measurements following the recommendations of the ICRU. These TLDs are loaded with carbon which absorbs all light emitted from anywhere in the crystal except the very surface. Therefore, only the light from a shallow surface layer of lithium fluoride (LiF) reaches the light detector in the TLD reader. This results in a dose reading for a very shallow depth depending on the carbon loading.

4.2 TLD Characteristics

The dimensions of black TLDs (Vinten) can be seen with other parameters in Table 8. As a comparison, the dimensions of a standard LiF ribbon (Harshaw) are given. The dimensions of the chips were measured by means of a caliper with digital readout (Starrett). The weight of the chips was determined using a microbalance (Mettler) and the density of each chip calculated from weight and volume.

4.3 TLD Methodology

4.3.1 FLDR Project

Skin dose measurements on patients treated on the FLDR project were performed using paired conventional lithium fluoride chips which were applied to the field centre on each patient and a build up of 1 mm of dental wax was utilised. Dose results were averaged and if the two readings differed by more than ten percent, the readings were repeated.

TABLE 8

	Standard ribbon	Carbon-loaded chip
Manufacturer	Harshaw[a]	Vinten[b]
Material	LiF: Mg, Ti, TLD 100	Teflon with LiF carbon-loaded
Surface shape	square	round
Dimensions (mm)	3.19 x 3.19	diameter 14.3
Physical thickness (mm)	0.88 ± 0.01	0.41 ± 0.01
Area (mm^2)	10.16 ± 0.12	161.3 ± 0.8
Weight (mg)	23.6 ± 0.4	103.5 ± 2.0
Density (g/cm^3)	2.64 ± 0.11	1.57 ± 0.08
Thickness (g/cm^2)	0.232	0.064
Dose response DR (nC/Gy)	347.8	32.0
Precision of dose measured using standards (2 SD)	3%	5%
Variability of DR between chips of one set (2 SD)	8%	10%

Table 8 Characteristics of the TLD chips used in the present study
[a] Harshaw Chemical Company, Solon Ohio 44139, USA
[b] Vinten Analytic Systems, Bedfordshire SG19 IRB, England

4.3.2 FHDR

A more sophisticated dosimetry approached was utilised in the FHDR study. Paired carbon-loaded TLDs were inserted into a tissue equivalent polystyrene holder and applied to the field centre on the patient's skin. No additional build up was utilised for these measurements. If patient geometry and field size permitted, two further paired conventional chips were utilised. Firstly as an additional check of the D_{max} of the applied megavoltage (6 MV field), a 1.5 cm layer of tissue equivalent was applied over 1 pair and to estimate dose to the basal layer, 0.7 mm of bolus was applied over an additional pair of conventional lithium fluoride chips. Again the paired results were averaged and if the two readings differed by more than ten percent, the reading were repeated. The measured doses were compared with estimated skin doses in this project, for the evaluation of the accuracy of surface entry dose. Entry doses

were open field surfaces doses measured by a Markus chamber on a solid phantom for 6 MV photons, and corrected by the Rawlinson surface correction (Rawlinson et al. 1992).

4.4 TLD Readout and Evaluation

The TLDs were read in two different types of TLD readers with contact planchet heating (Victoreen 2800M and Vinten Toledo 654 with automatic sample changer). The carbon-loaded chips are teflon based, and could not be annealed at 400°C as recommended for lithium fluoride (Driscoll et al. 1986). In addition, the carbon chips should be annealed under a nitrogen atmosphere to avoid oxidisation of the carbon, therefore a combined readout and annealing procedure was used. The TLDs were heated in the Victoreen reader directly to 350°C and kept at this temperature under nitrogen atmosphere for 45 seconds. In the Vinten reader, the chips were annealed after readout at 300°C for sixteen seconds, under a nitrogen atmosphere. For the physical tests, 10 chips were combined to one set where each chip was individually calibrated in the 6 MV X-ray beam of a medical linear accelerator (Varian, USA; Clinac 2100C). In these regular dose response checks, 100 cGy was given to the chips at the depth of maximum dose in a solid water (Radiation Measurements Inc. RMI, USA) phantom. Each chip was characterised by its number in the set and a sensitivity value which relates the individual dose response of the chip to the mean dose response of the set (Kron et al. 1993). Experiments were performed with pairs of chips. One pair out of the set of ten chips was irradiated under standard conditions to a known dose of the same order of dose expected for the other TLDs. The readings of the other chips were evaluated using the sensitivity values for each single chip (Kron et al. 1993).

Exposures of the TLDs were made in the 6 MV X-ray beam (D_{20}/D_{10} = 0.577) of the linear accelerator using solid water slabs (30 x 30 cm^2) of various thickness as phantom material. For X-rays, the accelerator was calibrated to give a dose of 100 cGy per 100 monitor units (MV) at the depth of maximum dose 1.5 cm (field size 10 x 10 cm^2, FSD 100 cm). The exit dose measurements were performed using a polystyrene slab phantom, (30 x 30 cm^2) for three separations, 7.9 cm, 13.2 cm and 18.5 cm. Clinical black TLDs consisted of a set of 30 chips, machine annealed in the Vinten reader. Chips were calibrated for 100 cGy at Dmax for 6 MV photons, and 660 kv output from an external beam Caesium unit. Both individual sensitivity and energy dependence were recalibrated at regular 3 monthly intervals. A check of the supralinearity was made for each energy used, and regular dose response adjustments were made for effects of supralinearity.

5. CONTROL STUDIES ON REFLECTANCE UNIT

5.1 Normal Individual

In order to test the daily reproducibility of erythema and oxygenation indices in a physiological sense, daily readings were taken on four paired abdominal points in one normal individual (female, age 40). Each of these points were 3 cm from mid-line and spaced vertically (Figure 26). A total of 20 consecutive daily readings of erythema and oxygenation indices were performed to test the dependency of each of the paired sites and their constancy on a day to day basis.

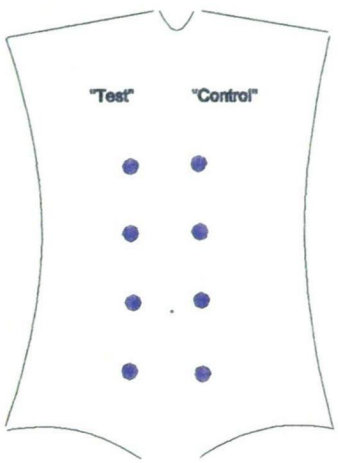


Fig 26 Schematic representation of the control study performed in a normal individual showing four paired measurement points analogous to the "control" and "test" points performed in the experimental situation.

5.2 Cancer Patients

In order to assess acute variation of erythema and oxygenation in relation to physiological parameters, a further control study was designed. The aims of the study were twofold;

1 To establish whether a radiotherapy field at a distant site (to the measurement point) had any demonstrable effect on haemoglobin or oxygenation indices. That is, to exclude a possible abscopal effect of the radiotherapy.

2 To demonstrate a possible relationship between haemoglobin, oxygenation parameters and other physiological variables. (systolic blood pressure, diastolic blood pressure, pulse rate and skin temperature).

Consenting patients were selected from the normal treatment population of the Department of Radiation Oncology, to conform as nearly as possible in terms of age, performance status and radiation dose to patients on the FLDR and FHDR studies. The important distinction between this control study and the FHDR/FLDR studies was that measurement points were taken from a single mid-abdominal point which was required to be at least twenty five centimetres **outside** the treated radiation field. Measurements were taken under standard conditions on a twice weekly basis with each measurement being spaced at five to eight minute intervals over a half hour period. Synchronously with each erythema and oxygenation reading, the patients' blood pressure, respiratory rate, pulse and skin surface temperature adjacent to the measurement point (Medtel instruments) was performed. Three males and three female patients were enrolled into this study.

A second series of control studies was performed on patients having radiotherapy to abdomino/pelvic sites. On this occasion, measurement sites were chosen at a distance from the abdomen in sun-exposed areas. Thus, this situation represented the converse of the initial six control patients. Again, six patients were enrolled to this second study (three males, 3 female). Physiological parameters were not studied in this second series.

5.3 " Artificial Erythema Study"

In an effort to relate the performance of the reflectance unit to the grading ability of various clinical observers, a control study was designed, which involved the production of several artificial grades of erythema utilising flesh coloured acrylic plates which were superimposed on an arbitrarily designated light flesh colour. (to represent background skin) This enabled the quantification of reflectance measurements, with a linear increase in red pigment concentration and an estimation of the performance of the human eye in the arbitrary grading of the erythema, both with respect to inter-observer variation and intra-observer variation.

5.3.1 Tile Construction

A light flesh coloured "background" tile (20cm x 10cm) was constructed by the local Dental Prosthetic Department using two standard prosthetic pigments (Ariabel Rose 300504-red and ICI Tioxide R-CR6-white). The best approximation to normal non sun-exposed skin was determined by trial and error by the two clinicians involved (CH and JD). This background slab had a 7cm x 7cm inset into which could be mounted "test" tiles. Fifteen further test

tiles were produced, with incrementally, linearly increasing concentrations of red pigment (range 0 - 2 mcg/Ml). Erythema readings were performed on each of the test tiles.

5.3.2 Grading Experiment

Under standard lighting conditions, five clinical observers (two consultants, two registrars and research nurse), were asked to visually grade the test tiles in succession, using the modified EORTC grading criteria from 0 to 2 inclusive. No time limit was applied and the clinical observers were not permitted visual reference to any other test tiles during the experiment apart from the large background "inset tile" upon which the "test tile" was laid. The grading experiment was repeated a day later in order to test intra-observer variability.

6 Data Collection and Statistical Analysis

All data was transcribed from individual patient data sheets onto a temporal database (Medlog, Information Systems Analysis Pty Ltd, California). Data quality assurance and descriptive statistics (means, frequency distributions, students T-test and Chi-squared tests), were also performed on Medlog. (Appendix 3).

6.1 Analysis of Normal Individual Reflectance Readings

In order to test for systematic differences in reflectance readings in the normal individual in both the superior-inferior and right to left direction, pooled means of four points on each day were subtracted and a students T-test performed comparing this difference to a constant (0). To assess the temporal pattern of reflectance values in the normal individual, two methods were employed;

1 Control Theory - pooled daily means (for each of eight points), were plotted against time and the overall mean (x) was also calculated. Control limits were taken above and below x as

$$\frac{3TSD}{\sqrt{8}} \qquad \textbf{(17)}$$

where

$$TSD = \sqrt{(SD_1)^2 + (SD_2)^2 + ...(SD_8)^2}$$

and SD_1.....SD_8 equal the standard deviations of pooled daily means.

2 All single point reflectance data was plotted against time and a polynomial smoothing algorithm employed to visualise significant trends with time (TableCurve, Jandel Scientific).

6.2 Analysis of Variables Effecting Erythema Response

Among the most important readily identifiable physiological factors potentially effecting erythema response are age, sex, site of measurement, skin type, tanning history and sun exposure. Our data clearly had several of these variables strongly correlated with one another. For example, males generally tended to have a higher rate of sun exposure and of course, sun exposure was also strongly site dependent. In addition, dose-related factors such as dose rate or dose per fraction were related to some of the physiological variables. Potentially important variables were first screened using uni-variate analysis. Variables which were significantly correlated (or nearly so) with peak erythema index on uni-variate analysis were included in a best sub-sets regression analysis (Sigmastat, Jandel Scientific). Variables with substantial multicollinearity were excluded from the model. The dependent variable (erythema index) also required a natural logarithmic transform to satisfy conditions of normality. Treatment site was modelled as four separate binary variables to eliminate further (artificial) numeric dependence with sun exposure. Other semi-quantitative variables (sex,sun-exposure) were modelled as binary or quaternary variables (skin type, hair, tanning history). Dose was modelled as the Extrapolated Response Dose (ERD) in order to incorporate the effects of dose rate/dose per fraction. (α/β = 10, $t_{1/2}$ =0.5 hrs)

6.3 Area Under Curve and Curve Fitting Procedures

The time course of individual patient reactions had cumulative area under curve calculations performed in one of two ways;

1 Trapezoidal rule - in this approach no intrinsic curve smoothing is applied and a sum of trapezoids defined by each data point allows the area to be calculated.

2 Peak function cumulative area - in this approach a peak function was fitted to each individual patient reaction (Extra Value Function n=94, Lorentzian Function n=15). (TableCurve:Jandel Scientific) The curve is then integrated to give the area.

In both methods x-min was taken as 0 and x-max as 80 days. Other data curve fitting was performed in TableCurve or SigmaPlot (Jandel Scientific) both of which use iterative non-linear regression processes to converge to a residual sum of squares.

6.4 Quantal Analysis Techniques

6.4.1 Binned Dose Response Plots

Raw FLDR peak erythema index dose response plots were converted to quantal probability plots in the following manner; The data set was divided using the median peak erythema index (1.6) as a cut point and measured dose data was binned into five to seven dose groups with cut points adjusted to ensure each bin included in the vicinity of ten to fifteen patients. The probability of response was then simply calculated as the probability of patients in each bin exceeding the median peak erythema. Standard error bars for each data point on the quantal plot, were given by equation 18:

$$SE = \sqrt{\frac{p\,(1-p)}{n}} \qquad (18)$$

where P equals the probability of response; that is of exceeding the median peak erythema index and n is the number of patients in each dose bin.

Binned dose response plots for the FHDR and prostate patients were derived in an identical manner with a peak erythema index cut point of 1.8.

6.4.2 Logit Dose Response Curves

For all iso-effect calculations (F_e, de Boer and Tucker plots) dose response curves were fitted by converting the peak erythema index data into quantal responses using cut points of:

1.5 (for comparison with Turesson's data)

1.6 (FLDR data analysis)

and 1.8 (FLDR, FHDR combined analysis).

$$P = \frac{1}{1 + e^{(a + bx)}} \qquad \textbf{(19)}$$

A variety of cut points were checked for each data set to test their effect on ED_{50} values. Quantal plots were fitted using a logit function, (19) where P equals the observed probability of response and a and b are constants and x is the quantal response. Logit fits were derived without dose-binning .

Modified F_e plots (see equation 15 Chapter 1) were constructed from ED_{50} points derived from quantal logit fits as above. de Boer and Tucker plots were derived from the same dose response data according the methods described by these authors. (de Boer 1988; Tucker 1984) Direct analysis of the data was performed using the ABest program on a Fortran Compiler (Nilsson et al. 1990). Effect per Fraction vs. Dose per Fraction plots were fitted using Table Curve, with a fit forced through the origin. (without dose-binning). These plots are displayed with binned data points for clarity of presentation only.

CHAPTER III RESULTS

SECTION A

1 CONTROL STUDIES

1.1 Normal Individual

1.1.1 Erythema Data

Figure 27 displays the daily pooled means and pooled right and left erythema values for 20 working days in a normal female individual. The overall mean of the data is 13.15 (standard deviation 2.36). Total range of values is from 7.5 to 20.7. It is readily appreciated that the daily mean of each of the eight measured points varies over a narrower range, from approximately 11 to 16 units. This figure also displays the pooled right and left measurement points for comparison. This figure suggests a systematic difference between the right and left side with right sided values almost always lower on average than left. This is confirmed with a Students T-test, (pair wise) with a mean difference of 1.26 units ($p < 0.001$).

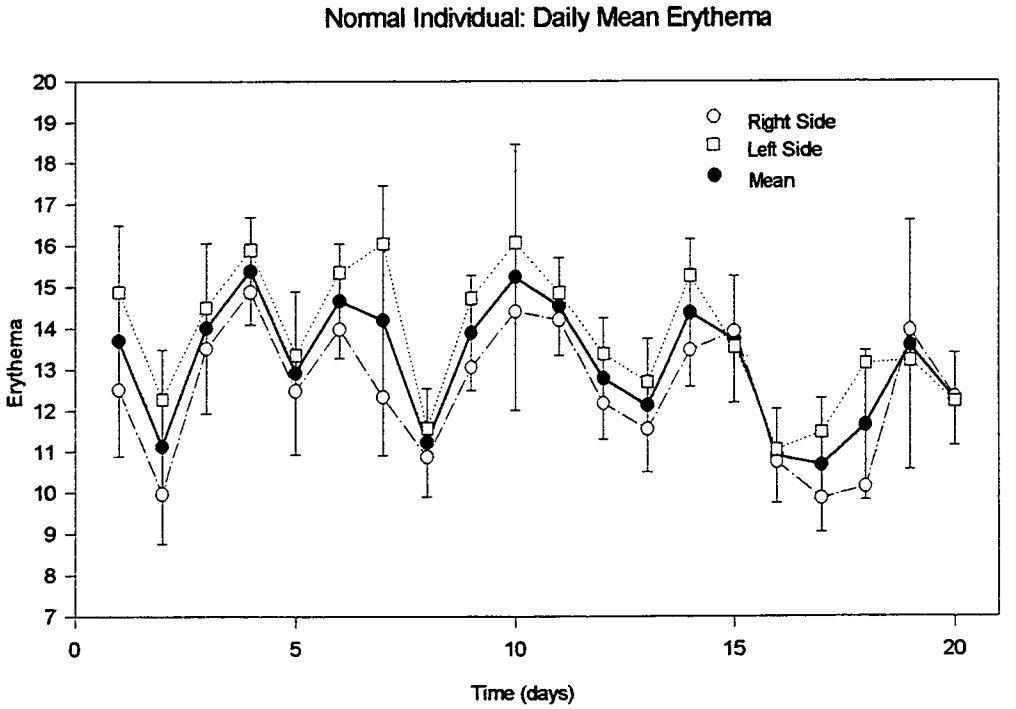


Fig 27 Daily mean erythema over 20 working days (± 1 standard deviation). Open symbols denote pooled right and left measurement points.

Figure 28 displays similar information, with on this occasion, superior and inferior groupings displayed with the mean data. Again a significant trend between superior to inferior is demonstrated with a mean difference of 1.19 units ($p < 0.001$).

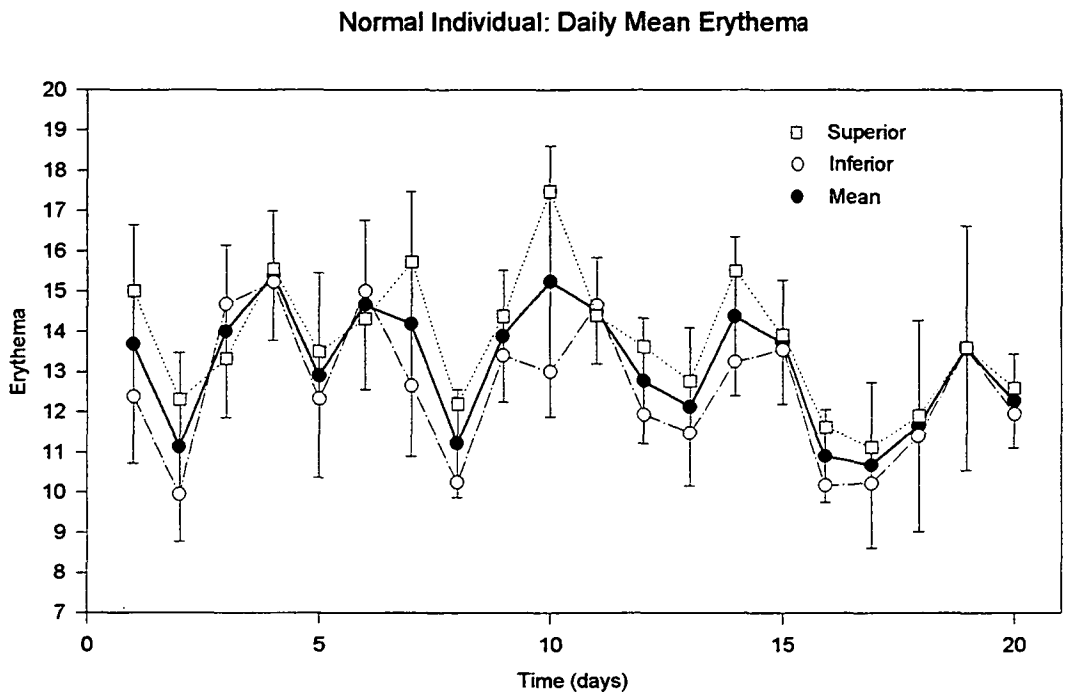


Fig 28 Daily mean erythema over 20 working days (± 1 standard deviation). Open symbols denote pooled inferior and superior measurement points.

1.1.2 Temporal Trends

Application of control theory to the results reveals that over the twenty days studied at no point did daily mean values exceed upper and lower control limits. This suggests no significant external influence or systematic variation in abdominal erythema values in the control subject. A polynomial smoothing algorithm applied to the raw data, also suggested no systematic temporal trends to this control data.

1.1.3 Oxygenation

Figure 30 displays mean oxygenation trends for the same individual. It is apparent that the range of oxygenation values and their reproducibility is substantially less than erythema. The overall mean was 17.44 (standard deviation 9.88). The range of values extended from -1.8 to 52.3. No significant trends with respect to laterality or superior-inferior position nor temporal variation could be determined.

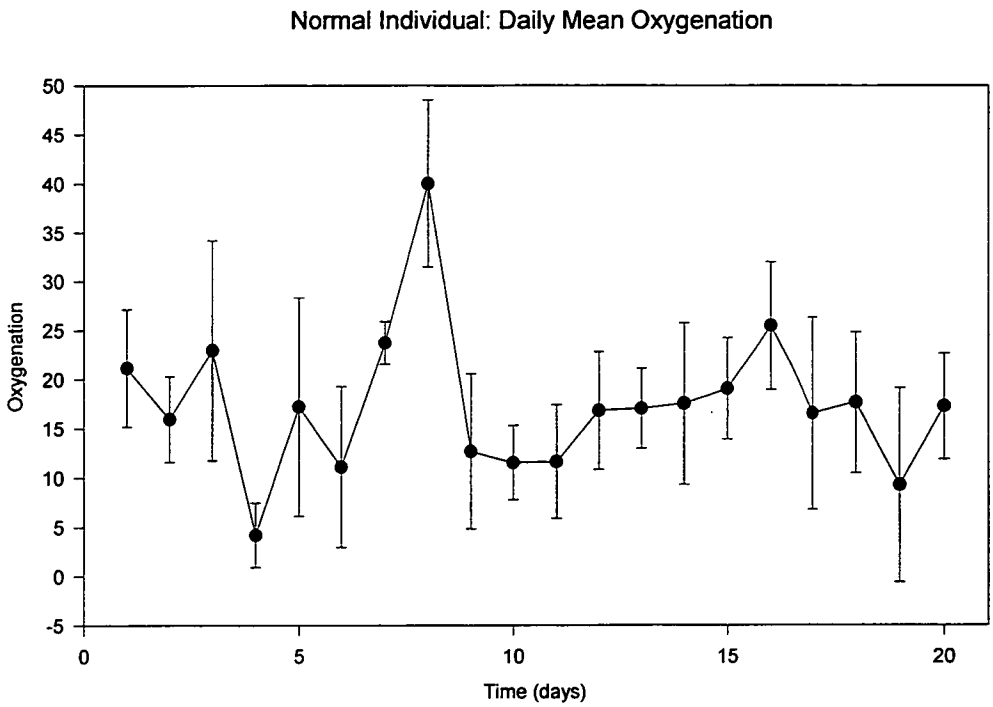


Fig 29 Pooled daily averages of oxygenation (± one standard deviation) over 20 working days.

1.2 Cancer Patients

1.2.1 Erythema Data

Figures 30 to 33 display raw erythema values (± one standard deviation) over a typical four to five week course of treatment for the radiotherapy control patients. Each figure describes the time course of raw erythema values over a 5-7 week period for each of 3 patients per figure.

Controls Sun-Exposed Females

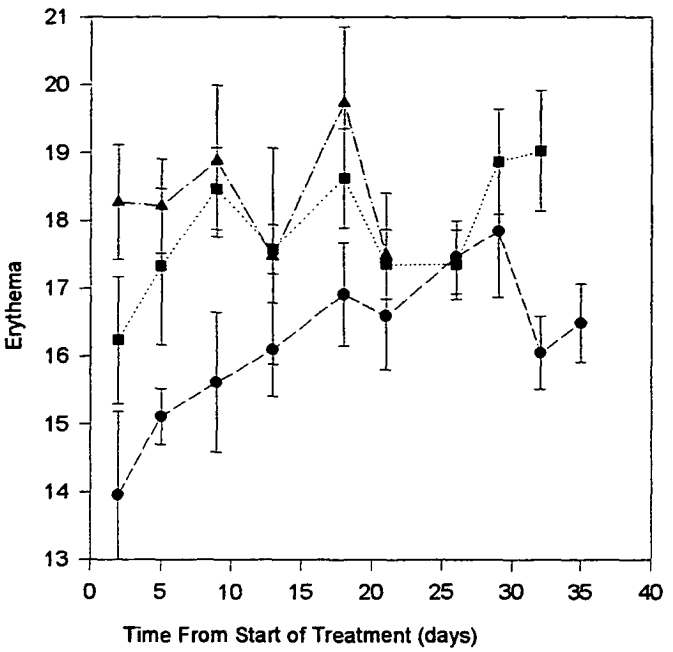


Fig 30

Controls Sun-Exposed Males

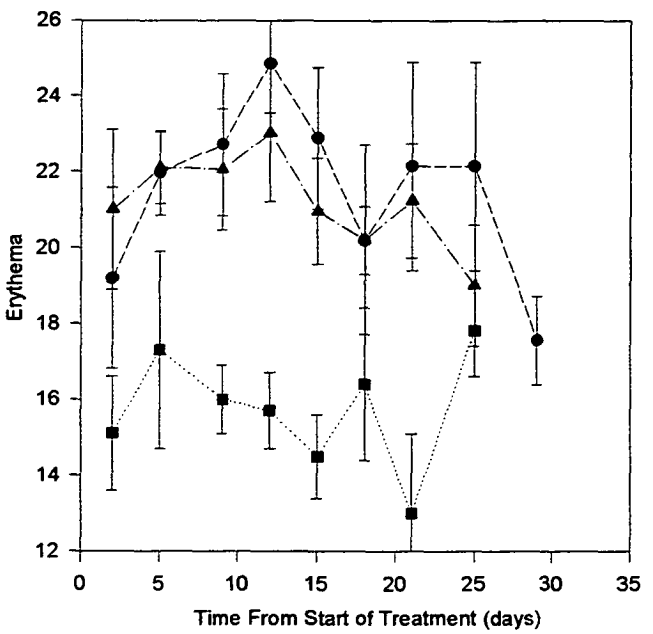


Fig 31

Controls Non-Exposed Females

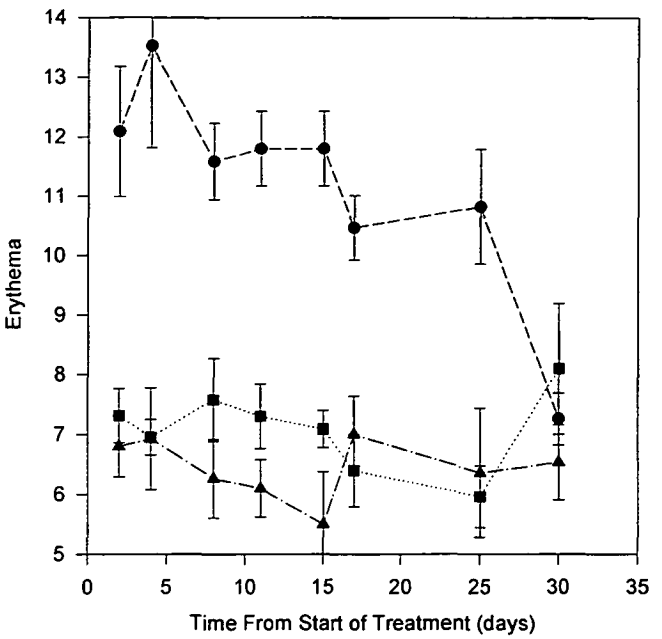


Fig 32

Controls Non-Exposed Males

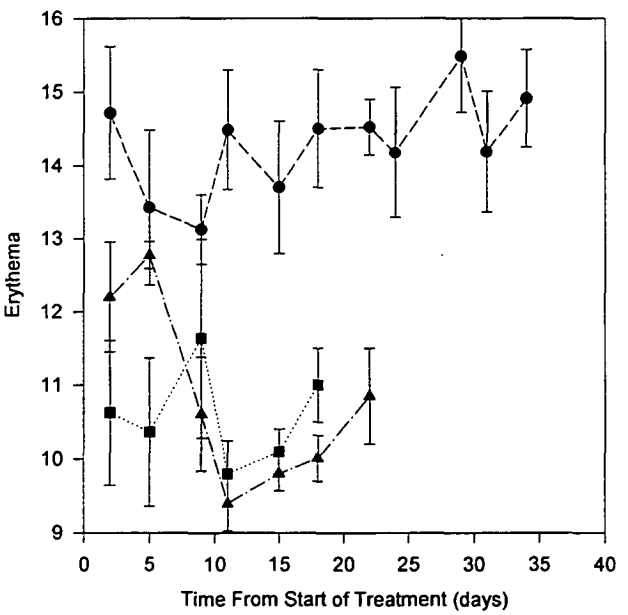


Fig 33

Fig 30-33 Mean daily erythema readings for control patients, grouped by sun exposure to measurement site and sex. Each symbol represents the mean of eight values, (± 1 SD) over an half hour period on each day on one patient. Figures 31 & 32 represent non-abdominal measurement sites and figures 33 & 34 abdominal measurement sites. (Radiotherapy delivered to a site **distant** to the measurement point)

With few exceptions, these trends show relatively steady state reflectance values over a relatively narrow and quite reproducible range. This data clearly show differences between individuals and suggests that males have higher baseline erythema readings than females in both non-exposed and exposed sites. The effect of previous sun-exposure on the measurement site is also evident with both higher values and greater variability, in contrast to the non sun-exposed skin.

1.2.2 Physiological Relationships

No relationship between erythema values and any of the physiological parameters studied, was able to be demonstrated. (systolic/diastolic blood pressure, pulse rate, respiratory rate and skin temperature) A poorly defined, but statistically significant inverse relationship was found in the cancer control patients, for skin temperature and oxygenation (figure 34).

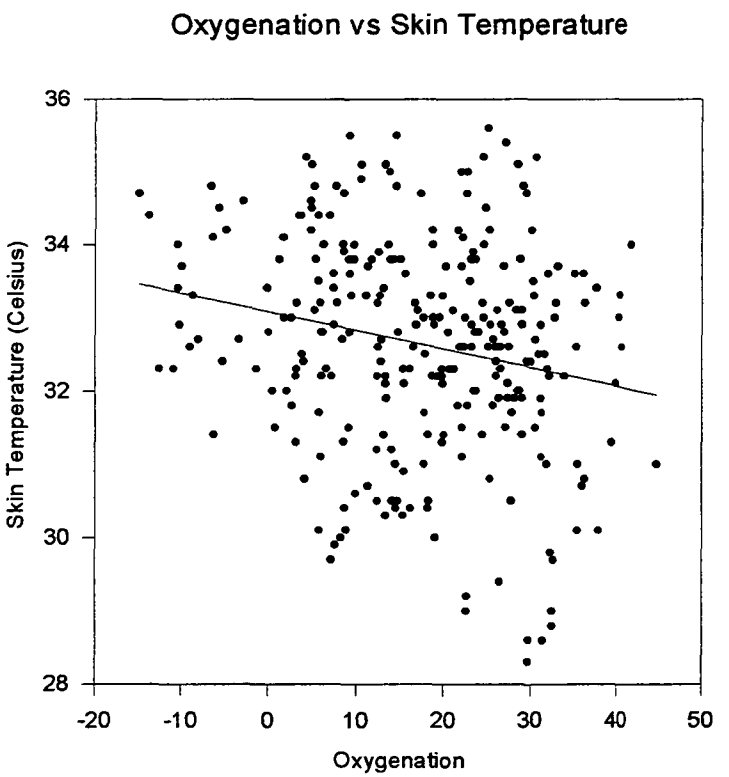


Fig 34 Skin temperature versus oxygenation for cancer control patients (n=6) (r^2=0.045) (probability that slope=0, p=0.003) (probability that intercept = 0, p=<0.0001).

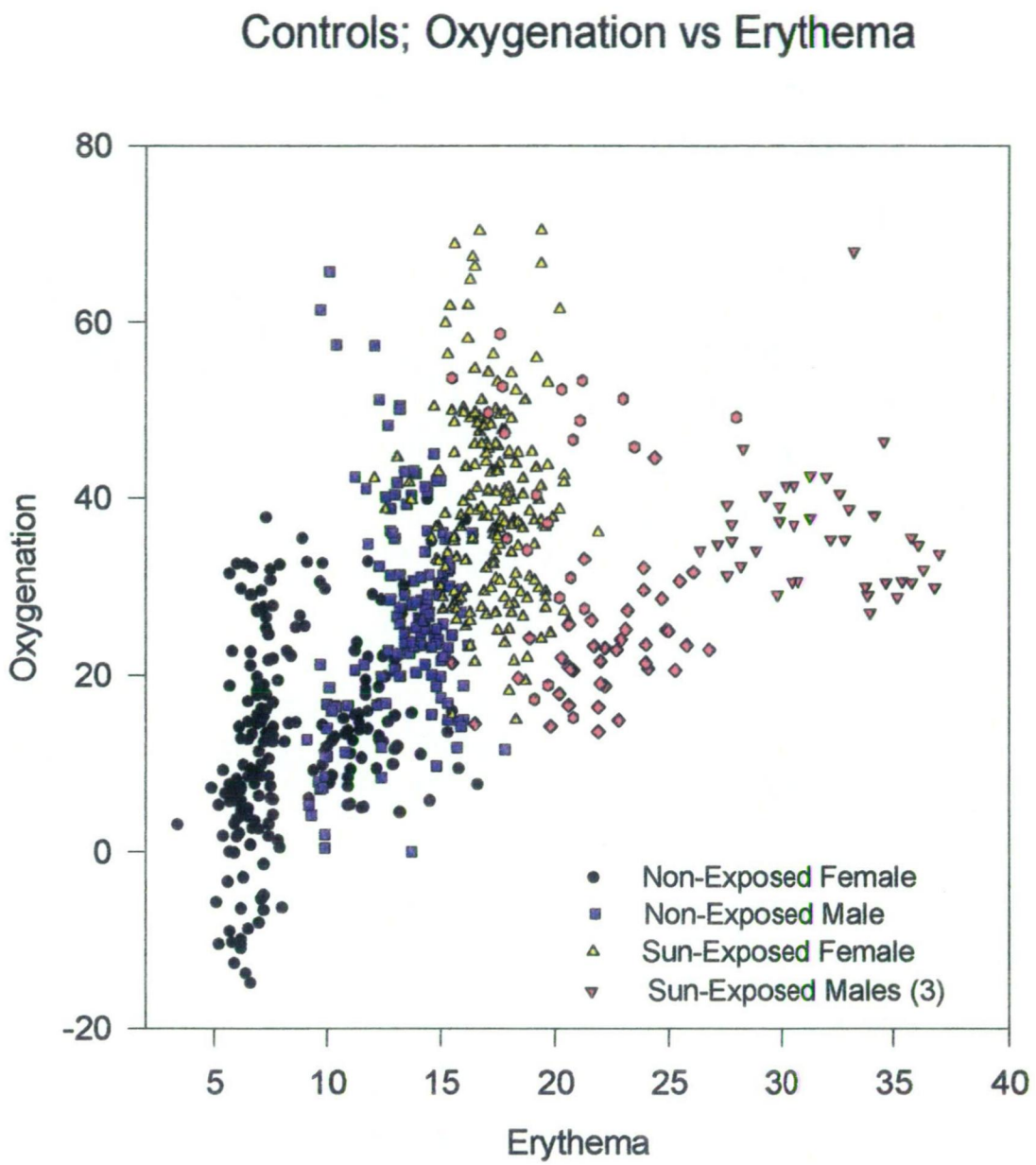


Fig 35 Oxygenation versus erythema for cancer control patients (n=6).

A relationship is demonstrated in the same group of patients, between oxygenation and erythema values (figure 35). This figure displays clustering of erythema values for certain patients (cf. figs 30-35) and patient groups (based on sex and previous sun-exposure) and an overall positive correlation between oxygenation and erythema for the whole control population.

1.3 "Artificial Erythema Study"

Figure 36 demonstrates the relationship between measured erythema and concentration of pink pigment used in tile construction. Reflectance unit response is evidently linear with pigment concentration, until reflectance values of approximately 55 are encountered, when some supra-linearity may be present.

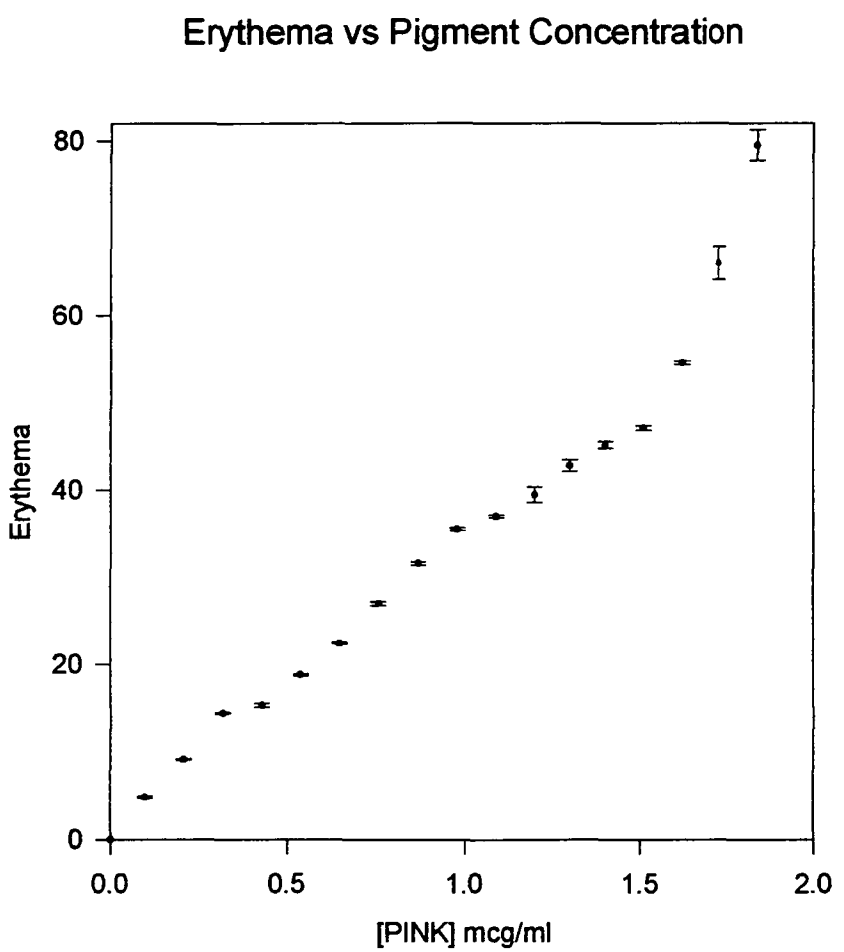


Fig 36 Concentration of pink "flesh equivalent" pigment versus measured erythema in acrylic tile. (error bars ± 1 SD)

Figure 37 shows the performance of five clinical observers in grading tiles of known pigment (and therefore known erythema) concentration. Clearly the range of overlap between grades is substantial, showing that the discriminatory power of the human observer in an individual case is limited. Figure 38 demonstrates the intra-observer variation of grade assignment and clearly suggests an even greater range of overlap between subjectively assigned grades of "erythema".

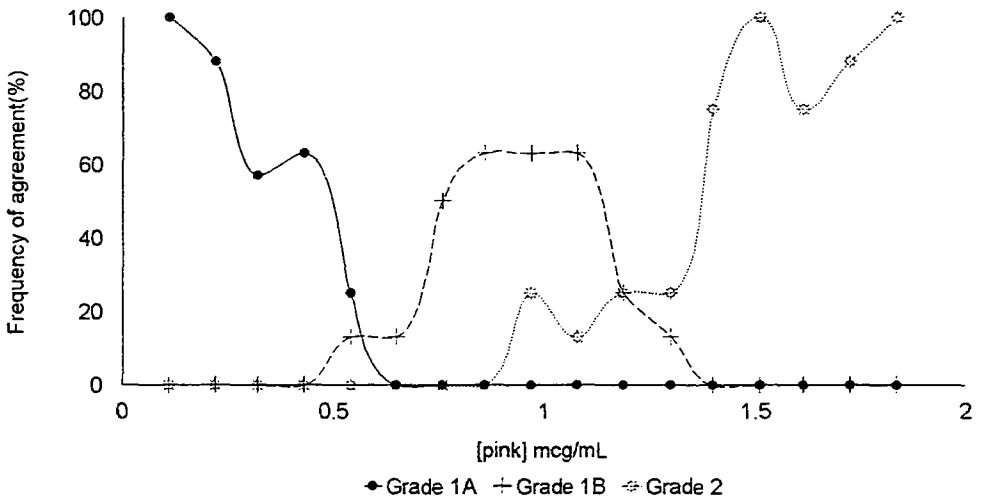


Fig 37 Likelihood of observer agreement (5 clinical observers) between assigned grade (0-2) and tile pigment concentration.

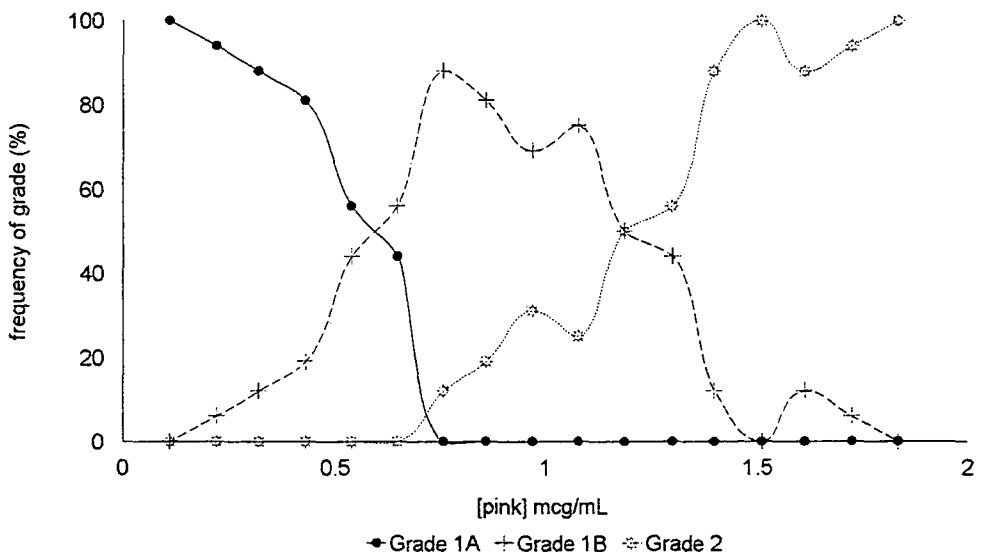


Fig 38 Probability of intra-observer agreement (day 1 vs day 2) for assigned grade (0-2) versus pigment concentration.

SECTION B FLDR

2 CLINICAL GRADING OF ERYTHEMA (FLDR)

2.1 Correlation

A similar exercise can be performed for the entire FLDR study (919 synchronous clinical grading and reflectance values). Table 9 reveals a clear linear correlation between observed erythema grades and measured continuous erythema values. This table demonstrates that females tend to have higher measured reflectance values at any given observed grade than males and a similar, even more pronounced trend is evident for non-exposed versus sun-exposed areas. General linear model regression analysis demonstrated that the slope coefficients differed significantly between males and females, non-exposed and exposed areas, and between the three major site classifications (Table 9)

TABLE 9

Groups (no. obs)	RHO	i'cept	p value	slope	p value	p value for equality of slopes	p value for equality of i'cepts
All ptts (919)	0.432	1.910	<0.0001	0.219	<0.0001		
Females (290)	0.587	1.235	"	0.341	"	<0.0001	=0.001
Males (629)	0.360	1.169	"	0.162	"		
Non-Exposed (278)	0.643	1.212	"	0.426	"	<0.0001	=0.055
Exposed (650)	0.364	1.173	"	0.153	"		
Site 1	0.267	1.156	"	0.097	"	1 vs 2 <0.0001	NS
Site 2	0.542	1.230	"	0.297	"	2 vs 3 =0.030	NS
Site 3	0.610	1.195	"	0.376	"	1 vs 3 <0.0001	NS
All ptts Oxygenation	0.077	-	NS	-	NS		

Table 9 General linear model regression analysis of reaction grade versus erythema index on the same day in the same patient (n=109)(site 1= head and neck, site 2 = upper torso, site 3 = lower torso, site 4 omitted due to low numbers).

2.2 Concordance

Figure 39 displays the relative frequency of recorded erythema indices at patient encounters when any given reaction grade was assigned by clinical observers. This figure demonstrates, in common with the artificial erythema study, a wide overlap of observed reaction grades in erythema index range. This spread of values for any given grade is more marked for the higher reaction grades.

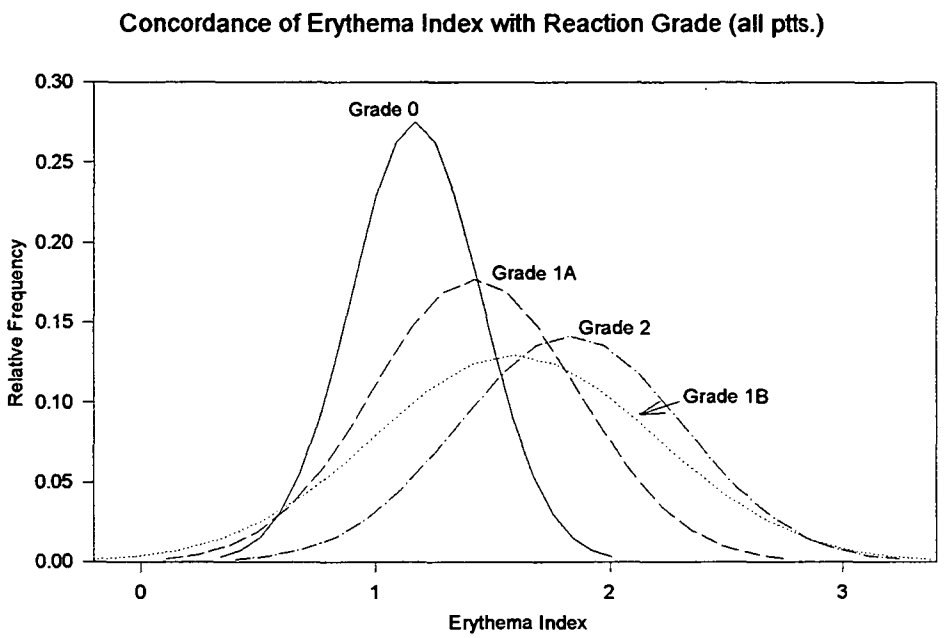


Fig 39 Relative frequency distribution of patient encounters at each of four observed grades (n=109). For demonstrative purposes, frequency histograms at each grade have been fitted with Gaussian functions (average RHO 0.65). (Grade 0 - 478 observations, Grade 1A - 280 observations, Grade 1B - 143 observations, Grade 2 - 13 observations).

3 VALIDATION OF ERYTHEMA INDEX (FLDR)

3.1 Usefulness of Control Erythema

Given the reproducibility and close concordance of anatomically adjacent points, with respect to erythema readings, the FLDR project utilised an adjacent control point just outside the treatment field to account for possible variation in baseline erythema during and after the treatment period.

3.1.1 Initial Values

Again, in common with the normal individual study, a high degree of linear correlation was found between the initial erythema reading in the test area and initial erythema control readings adjacent to the test area (n=109). Linear regression analysis reveals a slope of 0.827 with a probability that the slope=0 of $p<0.001$. RHO value for the linear regression analysis was 0.811. Mean initial test erythema value was 22.4 (± standard deviation of 8.4), and the mean value of the initial erythema control was 20.2 (± standard deviation of 8.44). (difference not significant on T-testing)

3.1.2 Inter- and Intra-patient Variation of Control Values

Table 10 demonstrates the mean control erythema values and coefficients of variation at an inter- and intra-patient level for the FLDR patients and the 12 cancer physiology control patients. Both these groups of patients clearly demonstrate significant differences in erythema readings related to both sex and sun exposure of the site under consideration. As previously demonstrated, males with sun-exposed measurement sites had higher baseline erythema with greater coefficients of variation. Both groups of patients demonstrated similar intra-patient variability with mean coefficients of variation of 12% and 11.9% respectively.

TABLE 10

INTER-PATIENT VARIATION		FLDR (n=109)		CONTROL (n=12)	
		Mean	CV	Mean	CV
MALES	Sun-exposed	20.6[a]	40%	25	22.3%
	Non-exposed	15.6[b]	34%	13.1	14.5%
FEMALES	Sun-exposed	17.4[c]	28.3%	17.5	8.6%
	Non-exposed	14.5[d]	31.7%	8.1	29.7%
ALL		19.5	40.5%	16.3	41%
INTRA-PATIENT VARIATION		Mean CV 12%		Mean CV 11.9%	
		Range 4 - 30%		Range 5 - 24%	

Table 10 Inter- and Intra-patient coefficients of variation (CV) for the FLDR group (**control readings**) and the physiology cancer control study group. The FLDR group had an average of 8.5 sequential readings per patient and the physiology control group an average of 62 readings per patient. Students T-test for mean erythema values FLDR patients; AD<0.001, BC<0.001 and CD<0.001.

3.1.3 Effects of Treatment on Control Values

If **all** compared test and control values for the FLDR study are correlated (n=908), then a less marked but still statistically significant correlation exists (slope=0.74, probability that slope=0 of $p<0.001$), and a RHO value of 0.602 ($p<0.001$). That is, despite the perturbing effect of treatment, a strong correlation exists between each test and control reading. This is displayed graphically in figure 40 where pooled data and descriptive statistics for **all** control and test erythema data for each dose-rate group is presented. The means corresponding to each dose-rate group for both test and control areas are seen to be correlated. Erythema values are higher for the 0.8 Gy/h group and the 3 Gy/h group, as a consequence of the head and neck treatment sites involved in these dose-rate groups. This figure also demonstrates the very considerable spread of data for both the test and control sites for the FLDR patients.

The mean control value during and after treatment did not differ significantly from the initial value, apart from the patients treated at 4.8 Gy/hr ($p<0.001$), where a slight rise and fall of values with time, may be noted. (Fig 41)

Closer scrutiny of individual control values and their time course, revealed that some 23% of patients exhibited a clear tendency during their reaction, for control values to mirror the temporal pattern of test values. A typical example of this is displayed in figure 42. This subgroup of patients revealed no consistent trends with respect to sex, age, site, sun exposure, measured dose or dose rate in comparison with the parent FLDR group . Linear Regression Analysis of peak control erythema index also failed to demonstrate any dependence on dose, age, sex, site,sun-exposure or melanin.

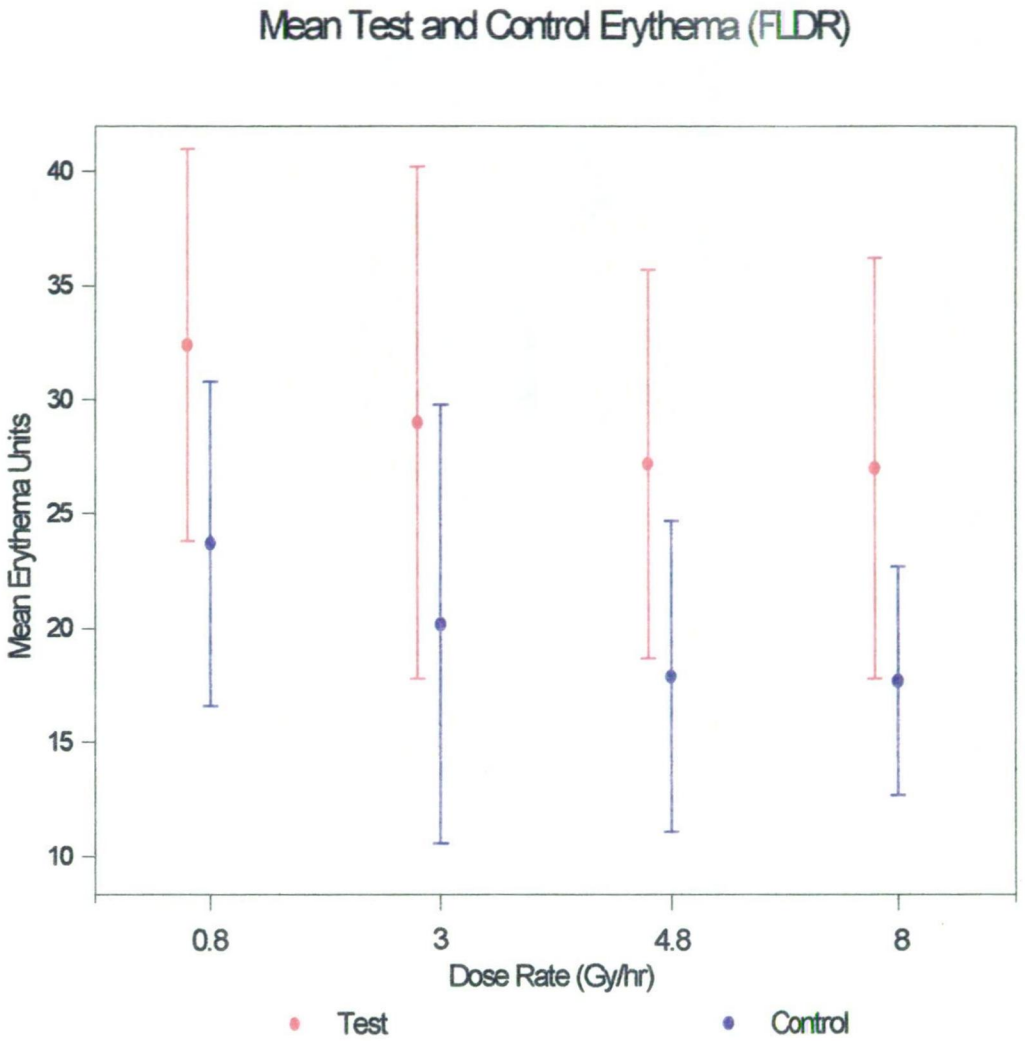


Fig 40 Mean Control (blue) and Test (red) erythema values (over time range 0-80 days) +/- one standard deviation for each of the dose rate groups.

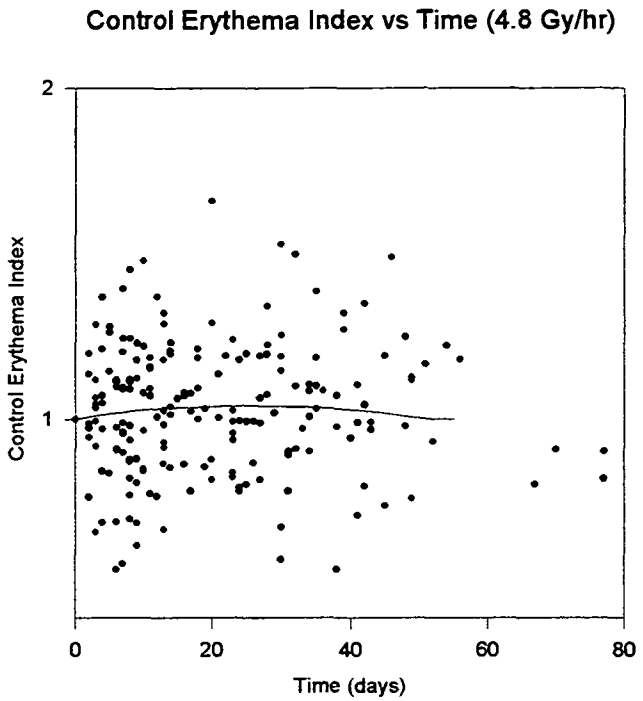


Fig 41 All control erythema data from start of treatment expressed as a normalised erythema index (Transform2, ref: Section4) for the 4.8 Gy/h dose group. Peak function fitted for demonstration.

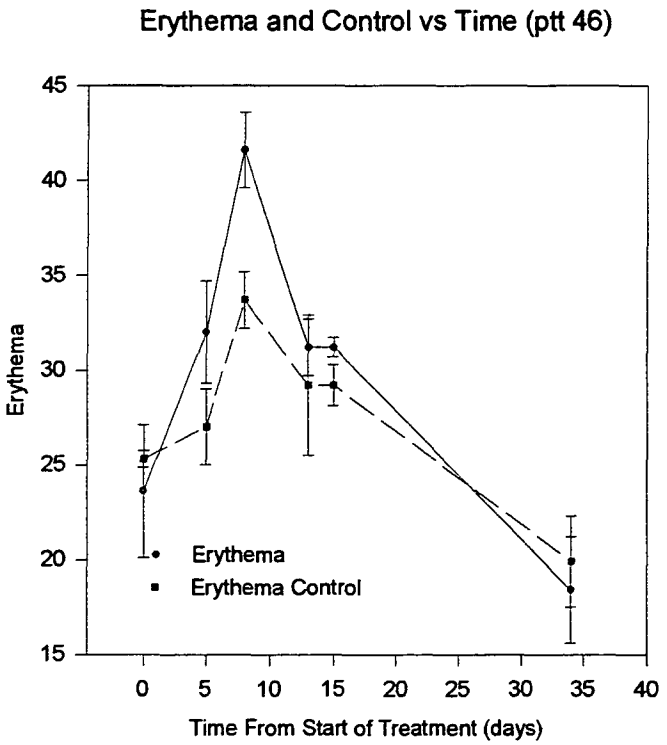


Fig 42 Example of control erythema "mirroring" test erythema.

4 DEVELOPMENT OF A SUITABLE ERYTHEMA INDEX

Five separate transformations were applied to measured erythema data in order to find an "erythema index" with the greatest utility for data analysis.

1 Raw erythema values

2 $$EI = \frac{E_t}{E_0}$$

3 $$EI_1 = E_t - E_C$$

4 $$EI_2 = \frac{EI \times E_C}{E_{C0}}$$

5 $$EI_3 = \frac{EI_1 \times E_C}{E_{C0}}$$

E_t = test erythema value at any given time. E_0 = initial test erythema. E_C = erythema control value at any given time. E_{C0} = initial erythema control value.

Each of these five transformations was applied to three areas of data analysis and the results intuitively and mathematically compared to assess the utility of each approach.

1 The time course of each individual patient's reaction was scanned by eye for each of the transforms above. Pooled data was also examined for goodness of fit of a suitable peak function to the time course data. Transform 2 provided the most cohesive plot, with the best fit of any given peak function (or by eye fit) in comparison to the other four methodologies. All other transformations produced noticeably worse fits, broader data spread and lower peak values.

2 The influence of age, sex, site and sun-exposure on peak erythema values was examined using each of the five transformations (linear regression modelling). The strongest and most consistent correlations were again demonstrated with method 2.

3 In a similar manner all five transformations were compared utilising raw dose response scatter plots. Again, the most coherent data set, with the best second order polynomial curve fits were achieved using the second transformation method.

4.1 Effects of Age, Sex, Sun-Exposure and Site on Initial Erythema (FLDR)

Univariate linear regression analysis demonstrated that age, sex, sun exposure and site all had important influences on the initial test erythema value for the FLDR patients. These trends reinforce results from the control patients, that is; males tend to have higher initial erythema readings as do sites in the upper part of the body. It is self evident that sex, site and sun-exposure are strongly inter-related, in that males tended to have higher rates of sun-exposure and heavily sun-exposed sites tended to be in the upper half of the body. Sub-set regression analysis confirmed these observations with significant correlation coefficients being demonstrated for sex ($p=0.0013$), site 2 ($p=0.0021$), site 3 ($p<0.0001$), site 4 ($p=0.0001$). Sun-exposure, age, site1 and initial melanin content were not significant. (site1 = head and neck, site2 = chest, site3 = lower torso, site4 =pelvis and others)

4.2 Effects of Age, Sex, Sun-exposure and Site on Peak Erythema Index (FLDR)

Sub-set linear regression analysis revealed age and sun-exposure to be significantly negatively correlated with the peak erythema index.($p=0.009$, $p=0.005$) Initial melanin content was negatively correlated with peak erythema. ($p=0.015$) Females displayed a higher response. ($p=0.03$) Biologically effective dose (ERD assuming an α/β ratio of 10 and a repair half-time of 1.5hrs.) and the adjacent control erythema were also strongly positively predictive of peak erythema index. ($p=0.006$, $p=0.0001$) For this analysis, site ceased to be significant. Field size was not significant. Regression analysis of peak oxygenation index revealed no significant influence due to the effects of site, sex, age, dose, melanin or previous sun-exposure.

4.3 Effects of Melanin

Melanin indices were assessed from spectrophotometry readings as a paired variable with each erythema index. Melanin index was recorded as an initial, peak and final value. Melanin index was found not to be significantly associated with initial erythema or skin reaction grade in linear regression analysis, however was negatively associated with the peak erythema index. In general, melanin indices did not alter in the same dramatic fashion as that noted for erythema. There was however, a small but statistically significant peak in melanin index in comparison to initial readings. This peak generally tended to be at or near the last of the erythema readings (mean pre-treatment melanin index 21.8 standard deviation ± 12.1, mean peak of melanin index 25.1 standard deviation ± 12.7, students T-test p value < 0.001).

5 TIME COURSE OF REACTIONS (FLDR)

The time course of observed and measured skin reactions in the FLDR study tended, in most instances, to follow a typical pattern. Reactions usually peaked between 0.6 and 1.5 months of starting treatment and had subsided completely by 1.5 to 2.8 months. Figure 43 displays the time course of reactions for the different dose rate groups. The higher dose rate groups clearly peaked higher and later than the 0.8 Gy/hr. group. The 0.8 Gy/hr. group reaction also returns to baseline well before the higher dose rates.

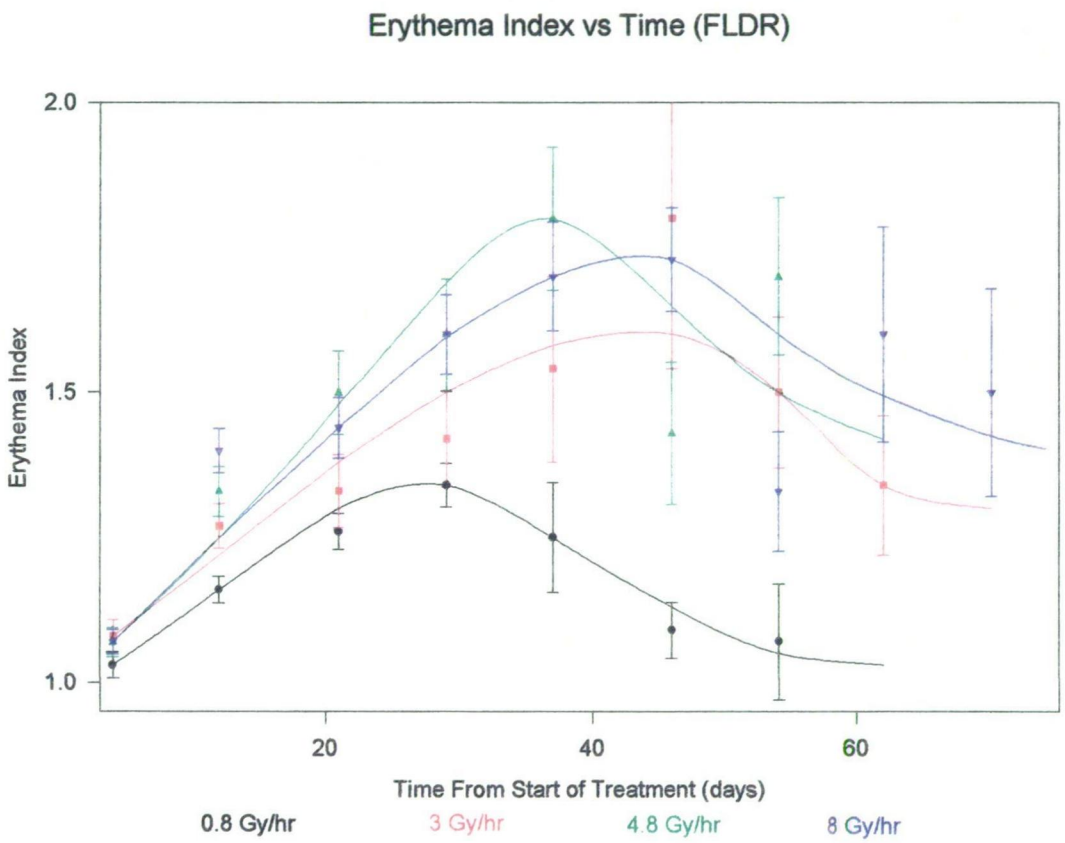


Fig 43 All FLDR patients reaction plots with time. Curve fits for each dose rate group by eye. Error Bars: +/-1 standard error. Elapsed time binned in 8 day intervals.

In 46% of cases graded skin reactions correlated well with the erythema index over the time course of the reaction (figure 44).

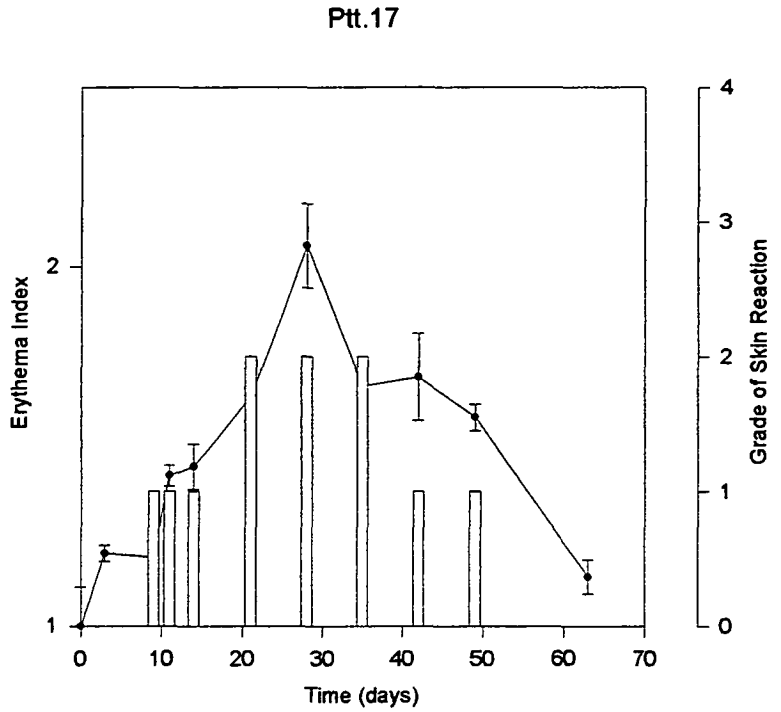


Fig 44 Typical patient reaction curve demonstrating close matching of measured erythema index and observed skin reaction grade with time

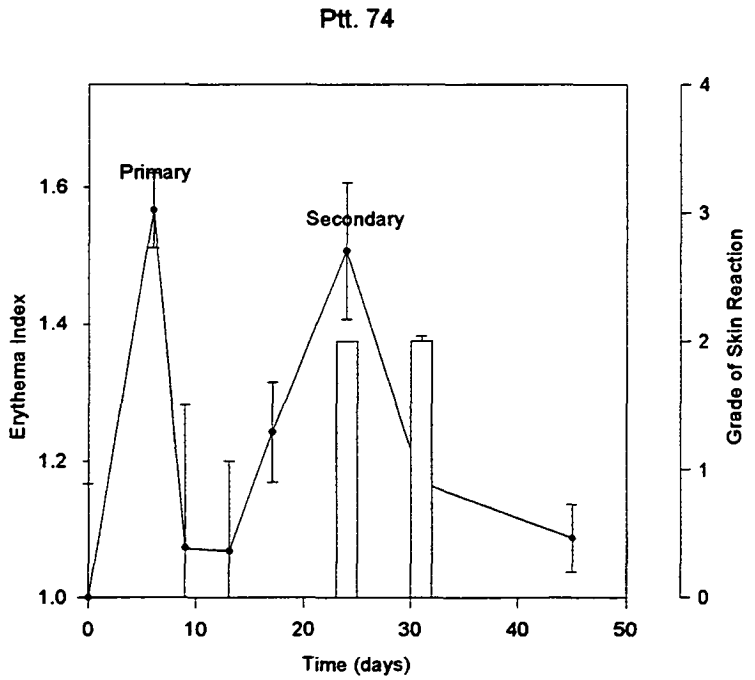


Fig 45 Typical patient reaction plot demonstrating clear rise in erythema index prior to observed erythema.

In 34% of cases there is a clear rise in the erythema index before any observable skin reaction and a return to baseline levels of both parameters simultaneously (figure 45). This figure also demonstrates a clear dip in the early portion of the erythema index reaction plot. This dip was usually noted during the second to third week of therapy and was seen in 34% of cases. The dip was not visible to the naked eye.

In a smaller number of patients (5%), the observed skin reaction clearly occurs before any measured erythema response is seen (figure 46).

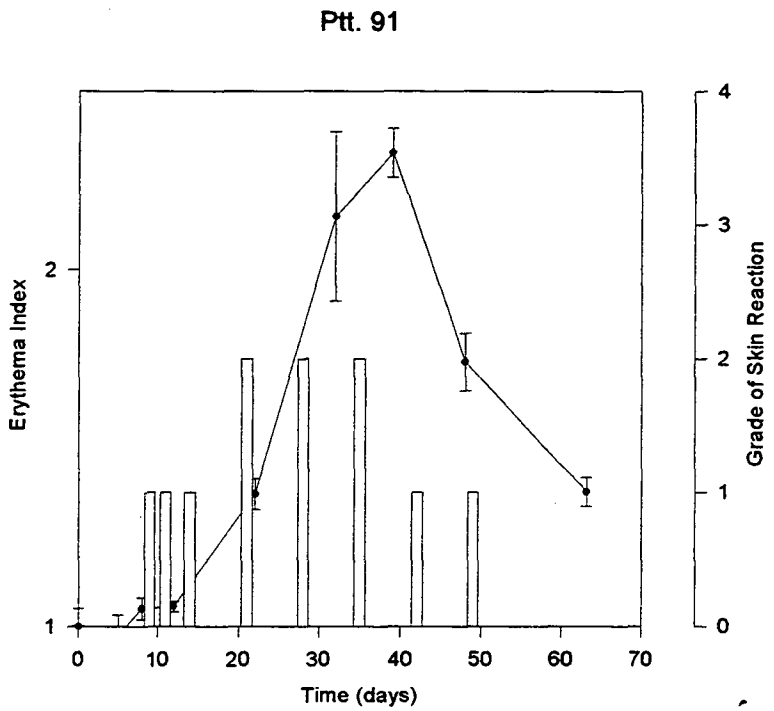


Fig 46 Typical patient reaction plot demonstrating a rise in erythema index prior to observed erythema.

In approximately 15% of cases no correlation between observed skin erythema and measured erythema could be discerned. This group of cases tended generally to exhibit no typical reaction pattern, with no clear rise or fall of measured erythema values.

6 TLD DOSIMETRY RESULTS (FLDR)

Figure 47 displays the prescribed skin dose for the FLDR patients versus the TLD measured dose. Because prescribed dose was specified to the mid-plane in the head and neck patients, there is a consistent 10-15% reduction in the measured skin dose versus prescribed mid-line dose. This is not seen in the skin patients with only a 1-3% under-estimate of prescribed skin dose versus measured dose in the 25-30 Gy region. Above 35 Gy the under-estimate rises to 3-5%.

A second consequence of the parallel opposed treatment field set up in the head and neck patients, was that each fraction of treatment was in fact given at two different dose rates when considering skin as the target organ. (this of course was not the case for mid-line mucosa). Entry and exit dose rates for the skin in this group of patients varied again by approximately ±15% from the specified mid-line dose rate. In the range of dose rates under consideration the dose rate effect is expected from the LQ predictions to be relatively minimal and a simple linear interpolation between the two dose rates produces an "effective" dose rate almost identical to a more formal LQ adjustment.

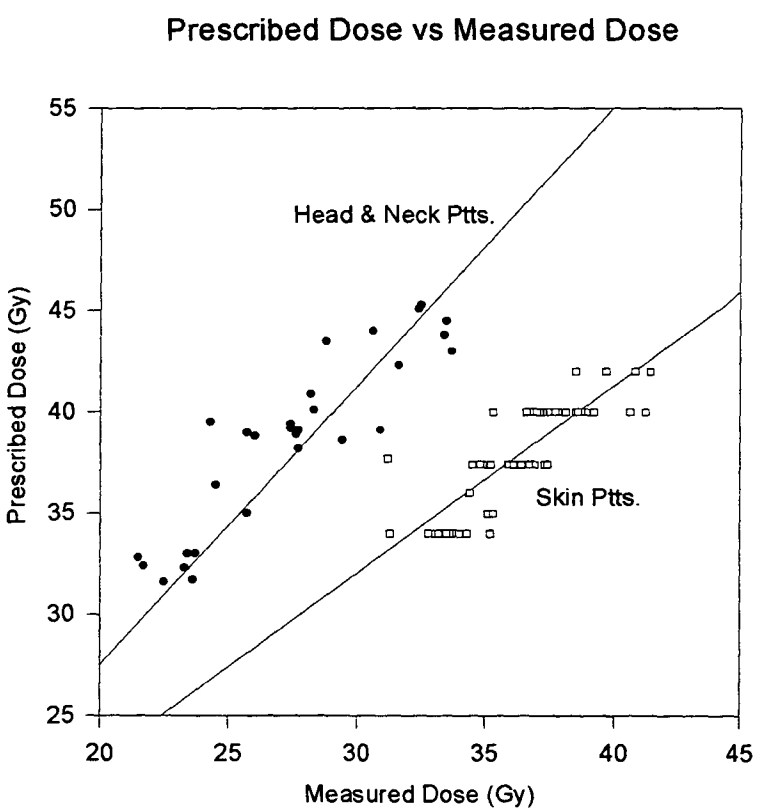


Fig 47 **Prescribed skin dose to FLDR patients versus the measured skin dose for each treatment group. Each treatment group is fitted by a linear plot.**

7 DOSE RESPONSE; DURATION METHOD (FLDR)

Figure 48 displays the relationship of duration of measured erythema at set levels above baseline, with dose. This figure displays a relatively wide spread of data, however, all four lines of best fit for each duration display similar slopes. Data has been presented on a semi-logarithmic plot for clarity and first order regression equations with confidence intervals fitted. The 95% confidence limits of D6 and D12 data overlap minimally suggesting these are the best two parameters of radiation effect in utilising the duration methodology.

Examination of durations of measured erythema for each of the different dose rate groups revealed closely superimposed lines with similar slope, (excluding 0.8 cGy/hr which displays a negative dose response plot), suggesting minimal impact of dose rate itself on the dose response curve using duration of erythema as response parameter over the range of doses in this study.

Separate dose response curves for males and females (using duration +6 and duration +12), display a trend for females to experience higher durations of erythema at both the D6 and D12 levels. In a similar manner a trend for greater durations of erythema for any given dose was seen in non sun-exposed treatment sites versus exposed treatment sites.

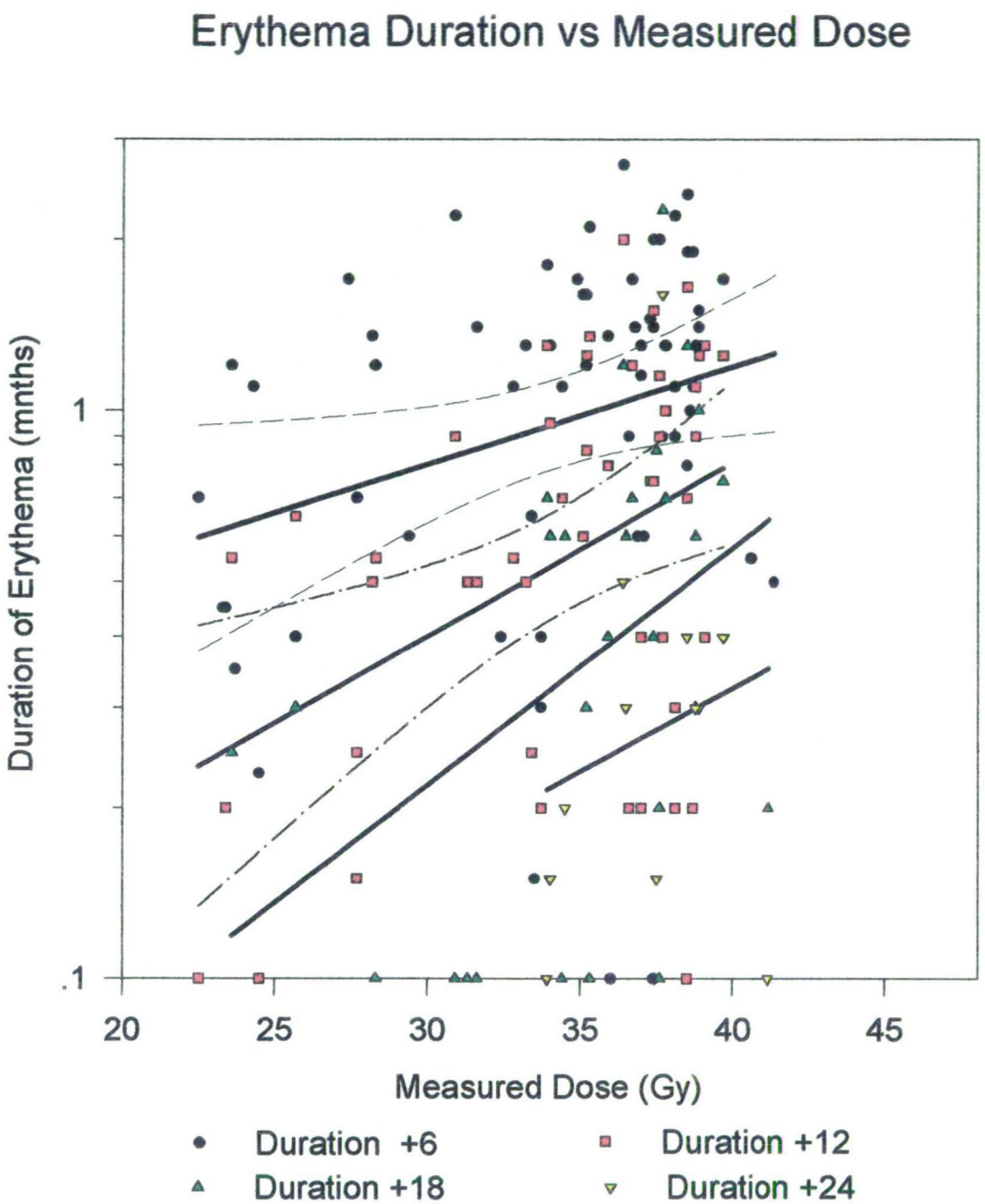


Fig 48 Semi-log plot of duration of measured erythema at 6, 12, 18 or 24 units above baseline for each individual patient versus the measured dose. Each duration "grade" is fitted with a first order regression equation with 95% confidence limits. D18 and D24 confidence limits overlap widely and are not displayed for reasons of clarity.

8 DOSE RESPONSE; PEAK METHOD (FLDR)

Figure 49 displays the raw peak erythema index data, for individual patients in the Head & Neck and Skin treatment groups, plotted against measured dose. A clear dose response trend is seen. In common with the duration methods, a marked but non significant difference in radiation sensitivity between the sexes is shown (figure 50).

Figure 51 displays the grouped anatomical sites and as indicated in the regression analyses, there appears to be an increased radiosensitivity associated with responses in site 2.

Figure 52 displays the apparent increased radiosensitivity in sites which have had previous significant UV exposure, however once again the confidence limits of these plots overlap.

Figure 53 displays the raw dose response plots for the individual dose rates employed in the FLDR study. Similar overall patterns are seen when compared to the duration plots and no coherent separation could be ascertained for any of the dose rates employed.

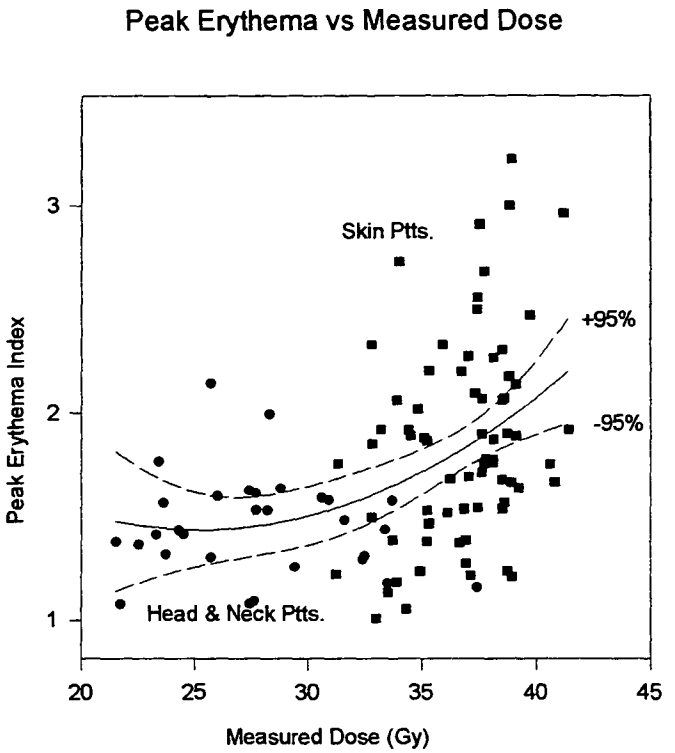


Fig 49 Peak erythema index plot for individual patients in the FLDR study versus measured dose (head and neck patients - circular symbols, skin patients - square symbols). Data best fitted by second order polynomial.

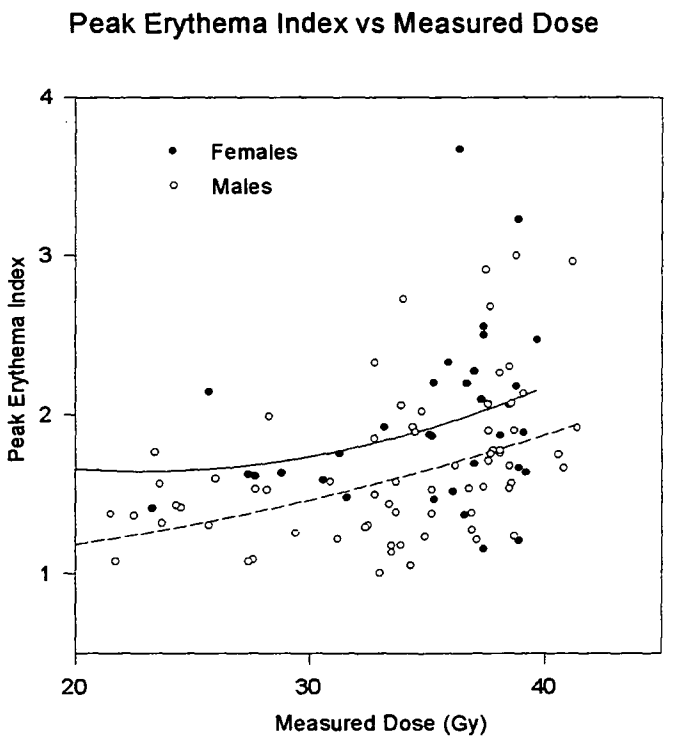


Fig 50 Peak erythema index versus dose for FLDR patients by sex. 95% confidence limits on second order polynomial fits overlap significantly.

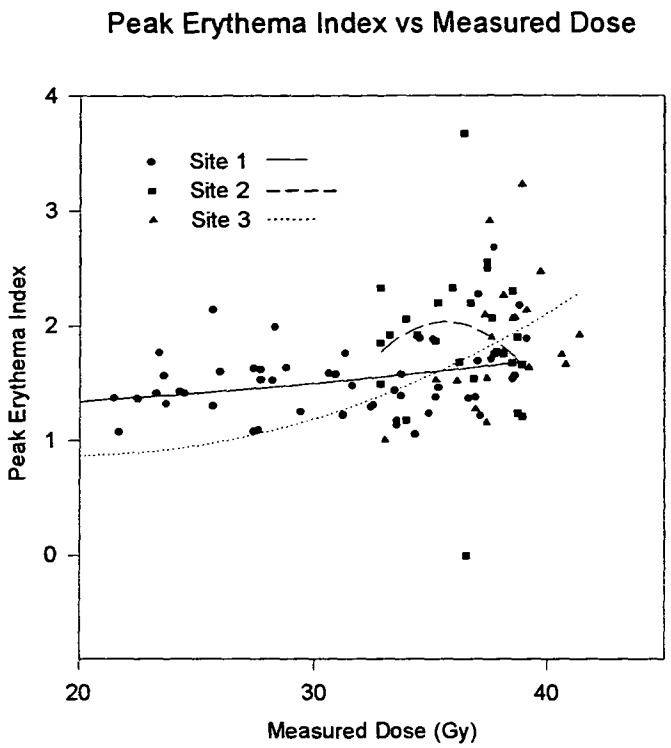


Fig 51 Peak erythema index versus measured dose for different body site groupings, site 1 = head and neck, site 2 = upper torso, site 3 = lower torso, site 4 excluded due to insufficient numbers.

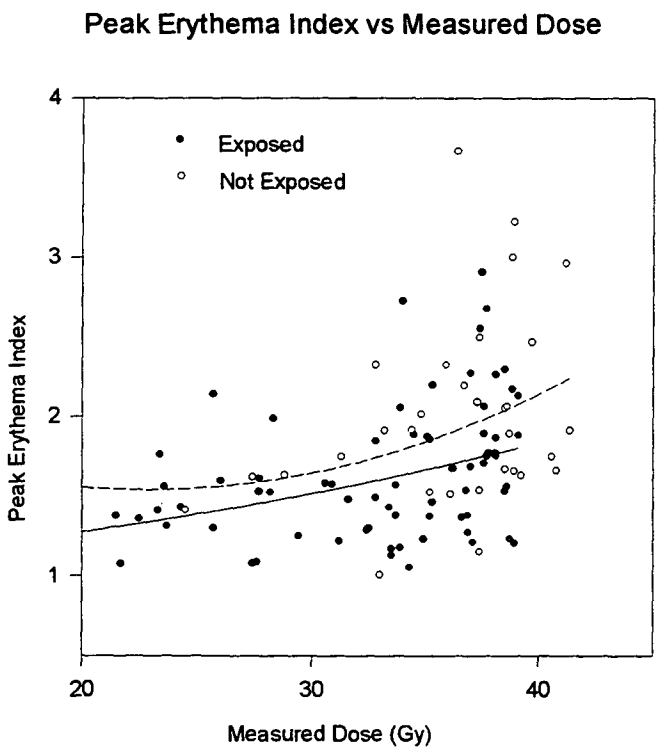


Fig 52 Peak erythema index vs dose for previously sun-exposed and non-exposed sites.

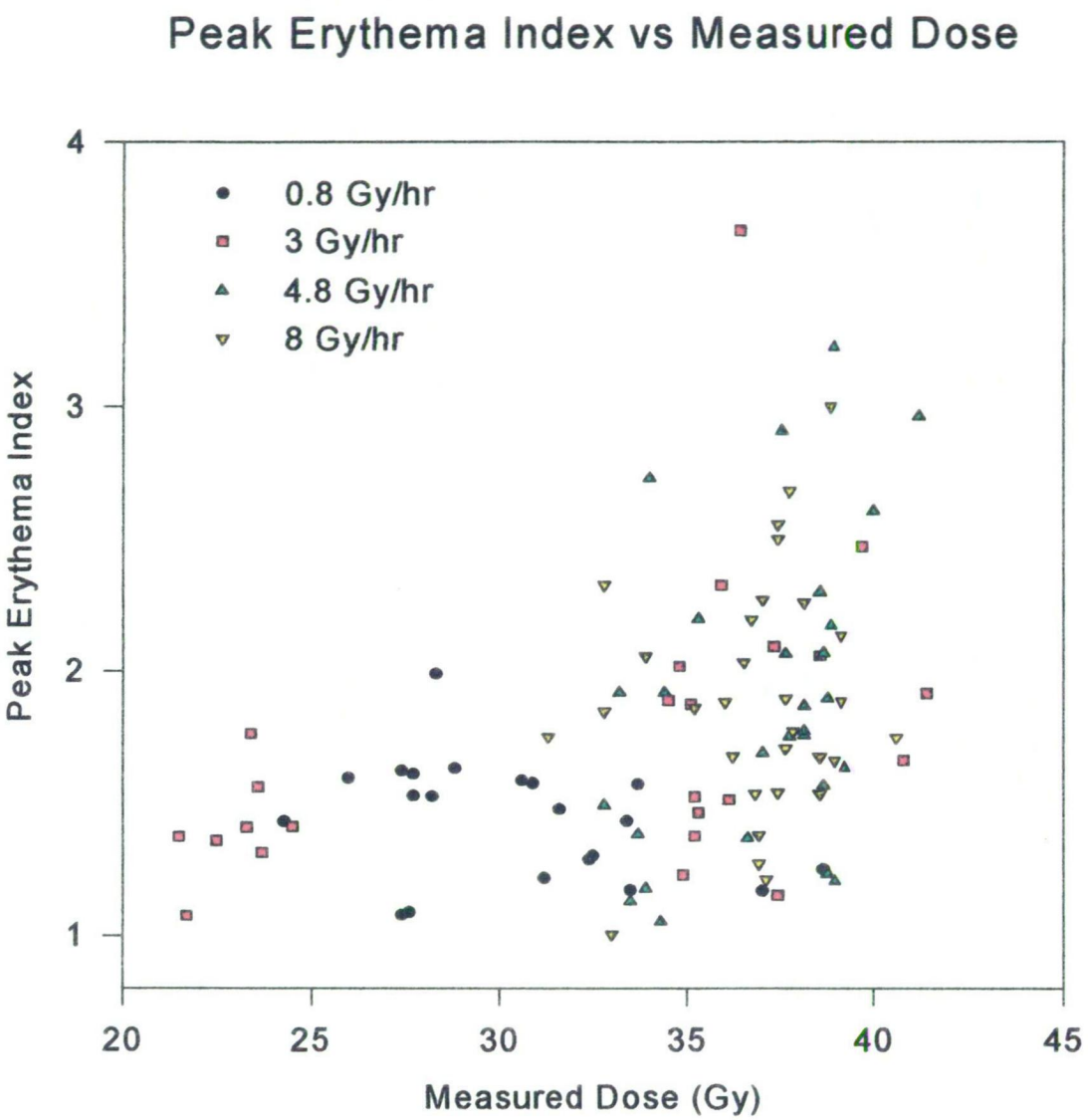


Fig 53 Peak erythema index by dose rate for FLDR patients against measured dose.

9 DOSE RESPONSE; INTEGRAL METHOD (FLDR)

Dose response plots using the individual patient "area under curve" values calculated for each patient using the "trapezoidal rule" and cumulative area function respectively were indistinguishable from the peak erythema index approach. This was confirmed when the integral erythema values were compared to the peak erythema index. Both were seen to be highly linearly correlated with an r^2 value of 0.83 for the trapezoidal method and 0.74 for the cumulative area function method.

Similar trends were observed with respect to sex, sun-exposure, site and dose rate differences in the integral erythema plot methodology when compared to the duration and peak methods.

10 RATE OF DEVELOPMENT OF ERYTHEMA (FLDR)

Figure 54 displays the relationship of the rate of rise of erythema for the FLDR patients plotted against the measured dose. There is only a weak dose response relationship and a suggestion that higher dose rates produce greater peak values, dose for dose. (cf. figure 43)

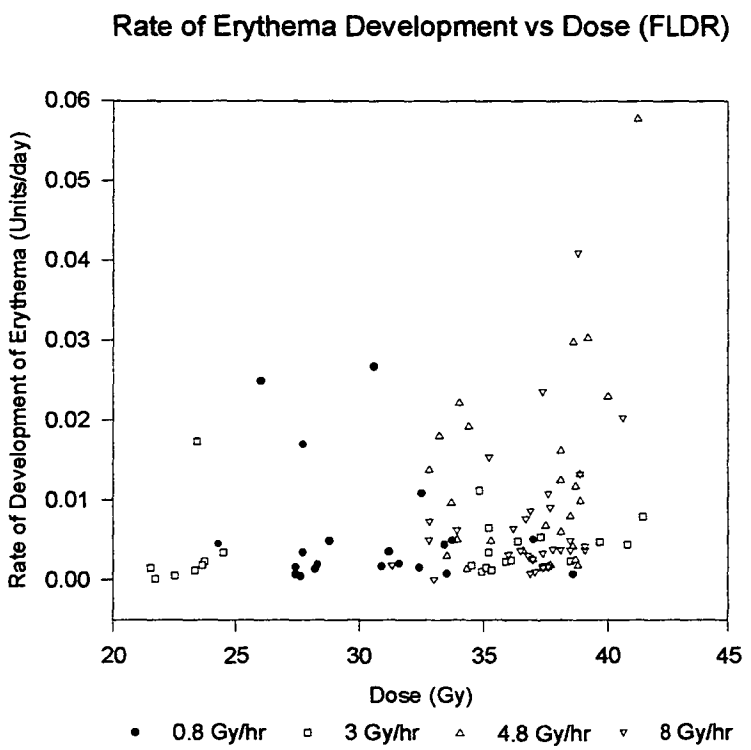


Fig 54 Rate of development of erythema in FLDR patients versus measured dose.

A more interesting relationship is demonstrated in figure 55, which relates peak erythema index in any given patient, to the rate of development of radiation erythema. Like the relationship of Peak to Area under Curve, there is a clear correlation between these two parameters, with significant linear correlation (typical correlation coefficients 0.3-0.5), below rates of approximately 0.05 erythema units per day. Although not statistically significant, there is a suggestion that higher peak erythema indices develop in association with higher dose rates (3, 4.8 and 8 Gy/hr). The only significant difference between rates of development of erythema for FLDR patients for each of the different dose-rate groups was in comparing the 4.8 and 8 Gy/hr group ($p<0.05$ Kruschal-Wallis)

Peak Erythema Index vs. Rate of Development of Erythema

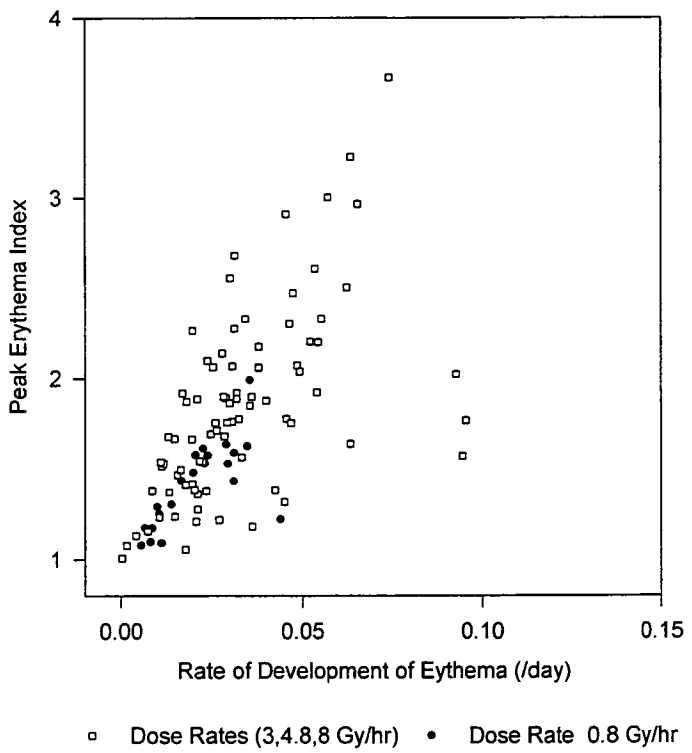


Fig 55 Peak erythema index for FLDR patients versus their rate of development of erythema. Higher dose rates have been pooled. First order regression fits through 0.8y/hr and pooled group.

11 DOSE RESPONSE; QUANTAL METHOD (FLDR)

The raw dose response data from the duration, peak, integral and peak erythema data may be converted into a probability response plot (see section 6 in the Materials and Methods section). Figure 56 displays the same data as fig. 53, but converted into quantal responses for the peak erythema index (method 2). No clear separation of dose response for each of the different dose rate groups could be discerned and therefore this figure uses pooled data for all the dose rate groups.

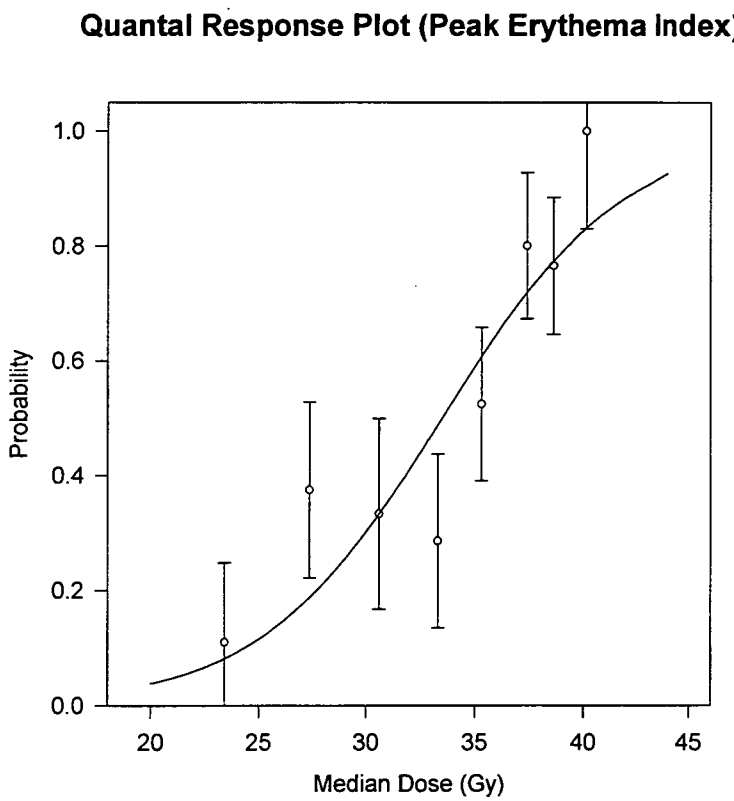


Fig 56 Logit fit through dose-binned quantal responses. (ie. the probability of peak erythema index exceeding the median value of 1.6, versus dose). (data is pooled for all dose rates)

Formal logit fitting of un-binned dose response data for the FLDR patients showed a negatively sloped dose response function for the 0.8 Gy/h group and marginally positive response for the 8 Gy/h group. (figure 57) Only the 3 and 4.8 Gy/h group produced reasonable fits with almost super-imposable sigmoid shaped curves and an ED_{50} almost identical to that of the pooled data seen in figure 56.

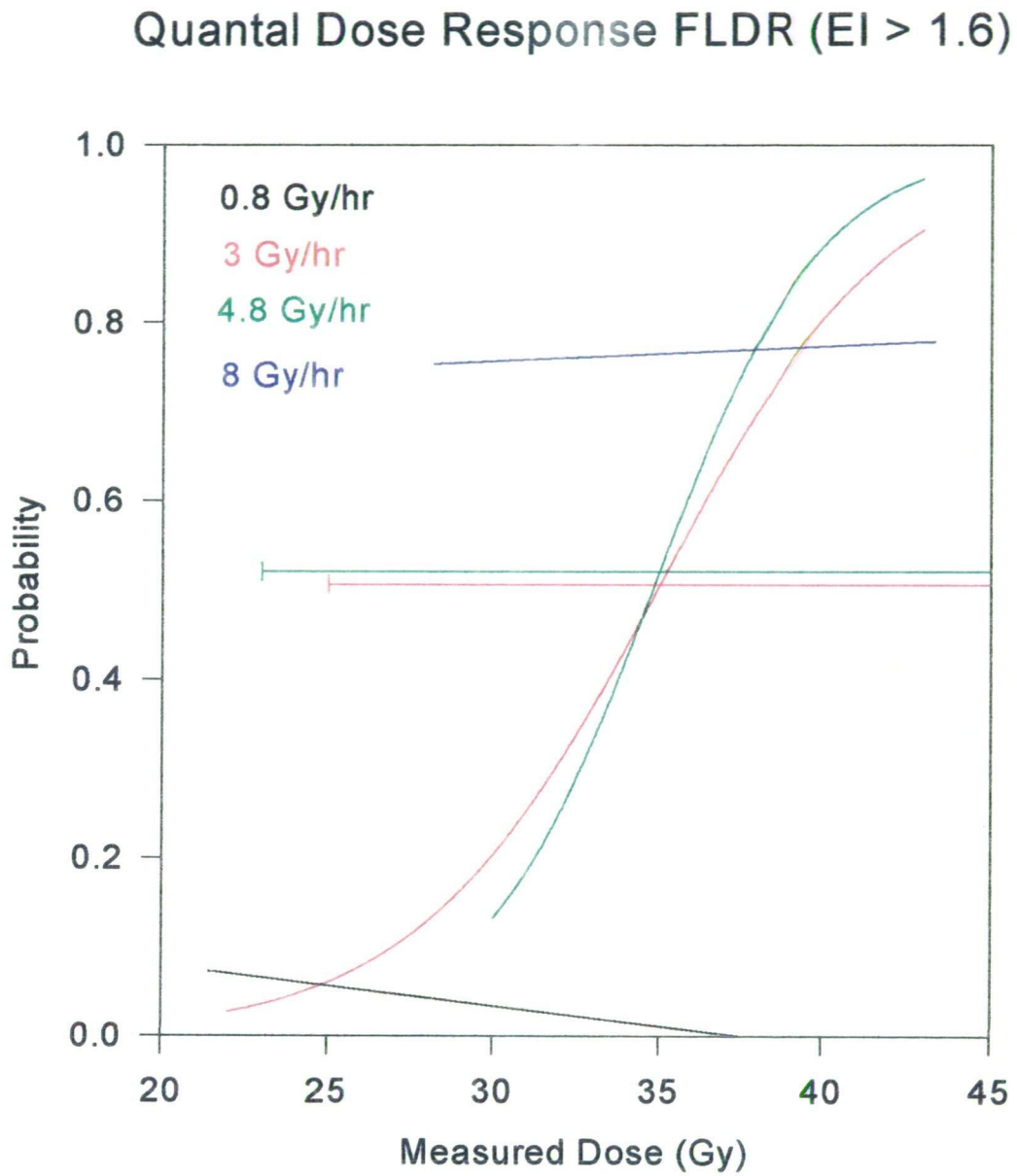


Fig 57 Logit plot of the probability of peak erythema index exceeding the median value of 1.6 versus un-binned dose for raw data (FLDR). 95% confidence limits displayed for the 3 and 4.8 Gy/h groups only.

Table 11 summarises the logit fits (RHO values and ED_{50} data) for a variety of acute erythema reaction parameters for the FLDR patients. This table confirms the by eye impression that the peak erythema measure produces the most coherent dose response measure. Peak and duration measures based on semi-quantitative reaction grading consistently failed to produce response plots with a positive slope.

Table 11

	RHO	ED_{50}
Peak erythema index	0.518	33.4
Integral erythema index	0.312	35.5
Duration 6	0.326	32.8
Duration 12	0.290	36.2
Peak oxygenation index	0.273	34.2
Rate of development of erythema	0.110	33.1

Table 11 Quality of logit fits for a variety of acute reaction parameters for the FLDR patients.

SECTION C FHDR

12 CONTROL ERYTHEMA VALUES

Intra-patient variability in the FHDR treatment group was similar to that in the FLDR group at approximately 14%. (Table 12). Inter-patient coefficients of variation were also in the 30-40% range,. It should be noted that the prostate patients exhibited an almost identical inter-patient coefficient of variation (33%) to the patients in the 5-20 fraction groups despite the wide variety of sites in those patients.

Table 12

	FHDR Prostate (n=51)		FHDR 5-20 fractions	
INTER-PATIENT VARIATION	Mean	CV	Mean	CV
Area 1	10.8	33%	16.6	36.8%
Area 2			14.3	34.7%
Area 3			14.8	40%
INTRA-PATIENT VARIATION	Mean Coefficient of Variation and (Range)		Mean Coefficient of Variation and (Range)	
Area 1	14% (4%-55%)		14.3% (3% - 55%)	
Area 2			14.6% (4% - 34%)	
Area 3			15.6% (1% - 57%)	

Table 12 Inter- and intra-patient coefficients of variation (CV) of raw erythema values for the FHDR group broken down into the hair cortical patients (32 fractions) and the remainder of the FHDR patients (5-20 fractions). The prostate patients have an average of 13.2 sequential readings/patient and the remainder of FHDR patients had an average of 9.5 sequential readings/patient.

Figure 58 displays the same data for both control readings and test readings in the FHDR patients. In common with fig. 40, there is heterogeneity of control and test erythema between groups. Similar causes exist for this variation. (*vide infra)*

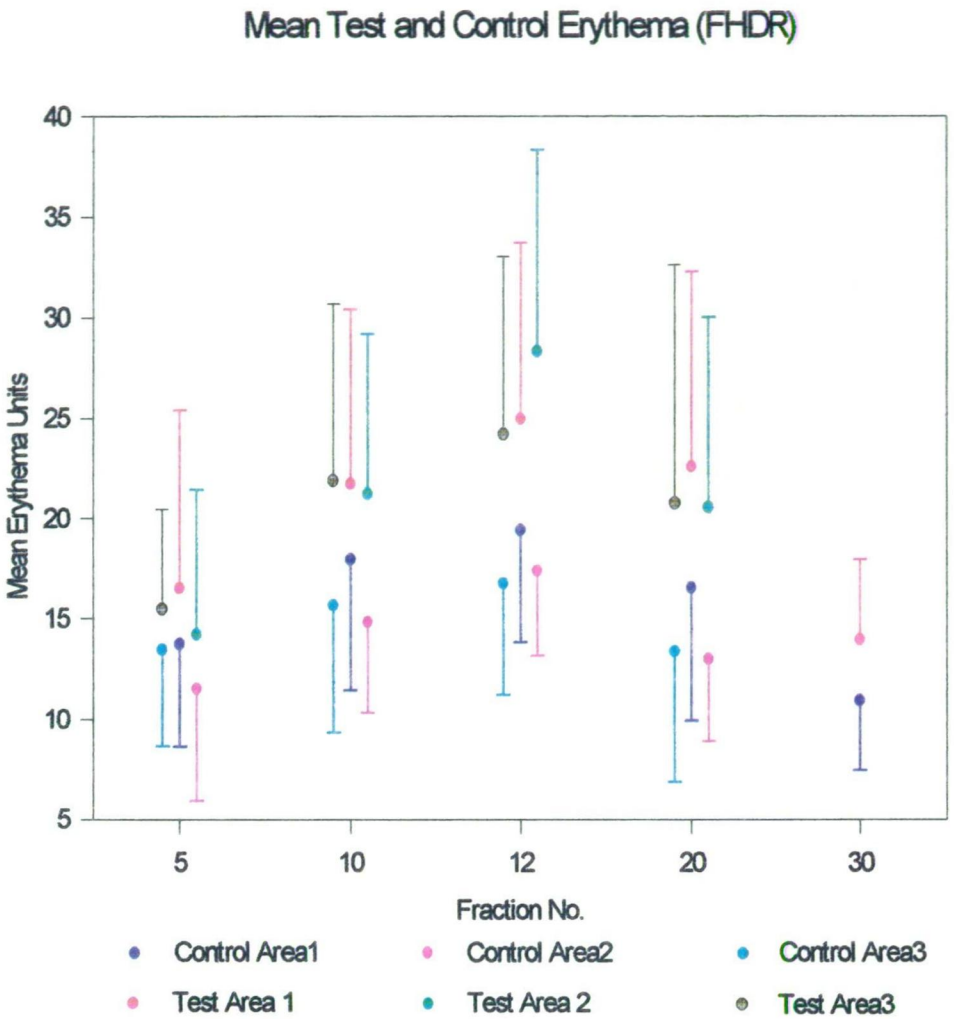


Fig 58 Mean and standard deviation of all test and control erythema values for each of the FHDR groups. Error bars are displayed in one direction for clarity.

13 TIME COURSE OF REACTIONS (FHDR)

13.1 In-field Reactions

In general, the pattern of rise, peak and fall of measured erythema values for the FHDR study mirrored that of the low dose rate study with the mean rates of development of erythema not significantly different in either study. Inspection of the raw erythema index versus time plots for each of the fractionation groups (5, 10, 12 and 20), revealed a slightly more heterogenous spread of data than the FLDR project. (figure 59) This figure also displays a general trend towards later peaks and more prolonged reactions with **increase** in fraction number. The rate of development of erythema was clearly higher for the five fraction group (0.127 units/day), however, this was not significantly different to any other FHDR or FLDR groups. The five fraction group receives, a high dose/fraction (median dose 4.4 Gy/fraction) and therefore a greater dose intensity/week. Again a subset of the patients also demonstrate a pronounced dip in their reactions at approximately 10-14 days from the commencement of treatment.

13.1 Out-of-field Reactions

In common the FLDR data set there was a subset of patients who displayed interdependence, or "mirroring" of control readings outside the field, with the in-field values. This is evident in figure 60 which displays plots from area 1 and area 3 in the one patient. For the first area there is a clear interdependence of the control readings with the test readings, however for the second area the control readings remain static while the test area demonstrates a typical radiation response. The FHDR out-of-field reactions peaked significantly earlier for the 5 fraction group, at approximately 20 days from the initiation of treatment. The rate of development of erythema for this group was significantly different to the rate of development of erythema for the hair cortical patients and the 20 fraction group (Kruschal-Wallis test $p<0.05$). (figure 61) The time course of out-of-field reactions in the FHDR patients showed considerable heterogeneity, however there was a general trend towards later peaks and more prolonged reactions particularly with **increasing** fraction number.

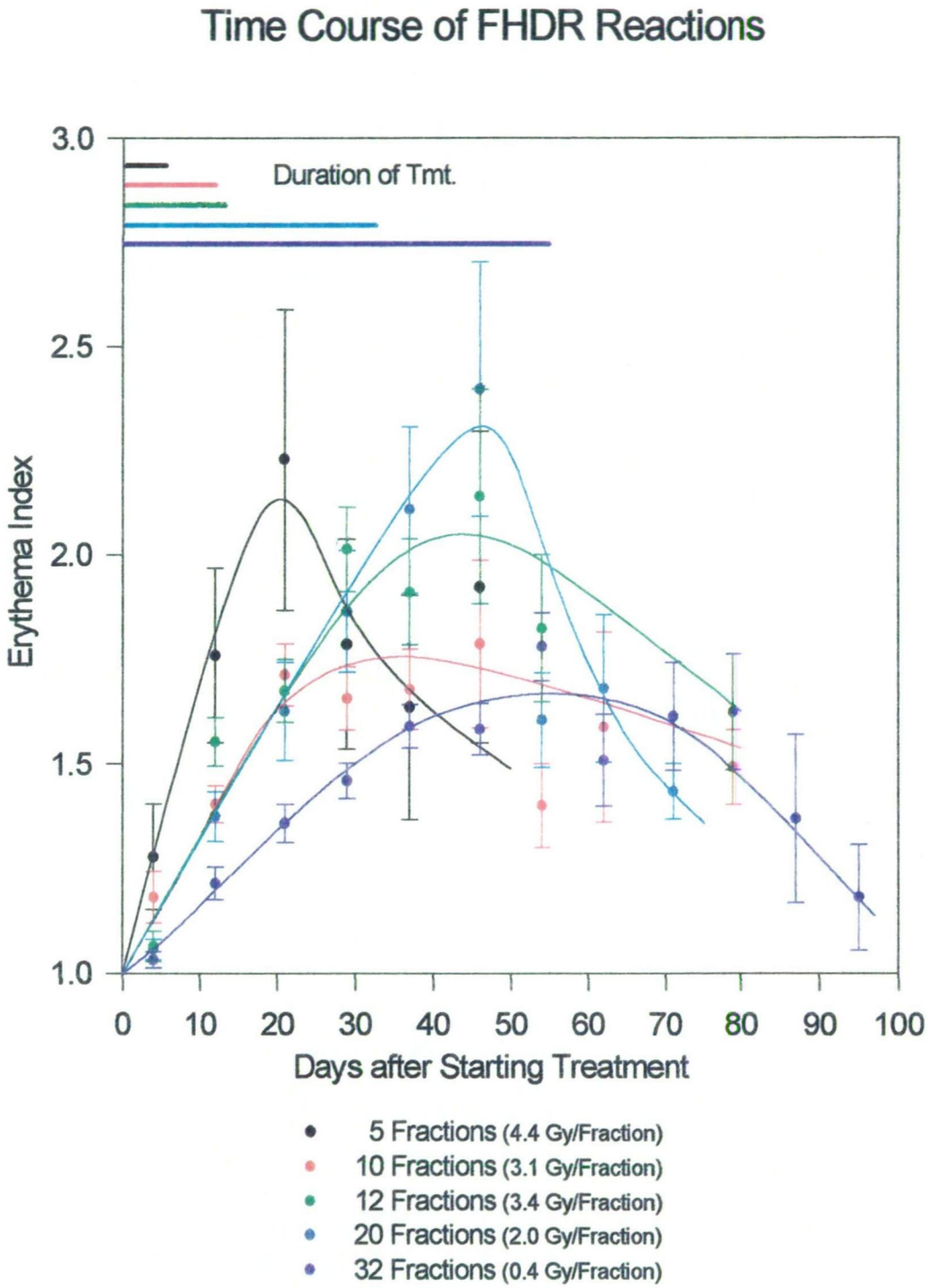


Fig 59 Time course of FHDR reactions. Best fits drawn by eye. Error bars: +/-1 standard error. Total duration of treatment displayed graphically at the top of the figure.

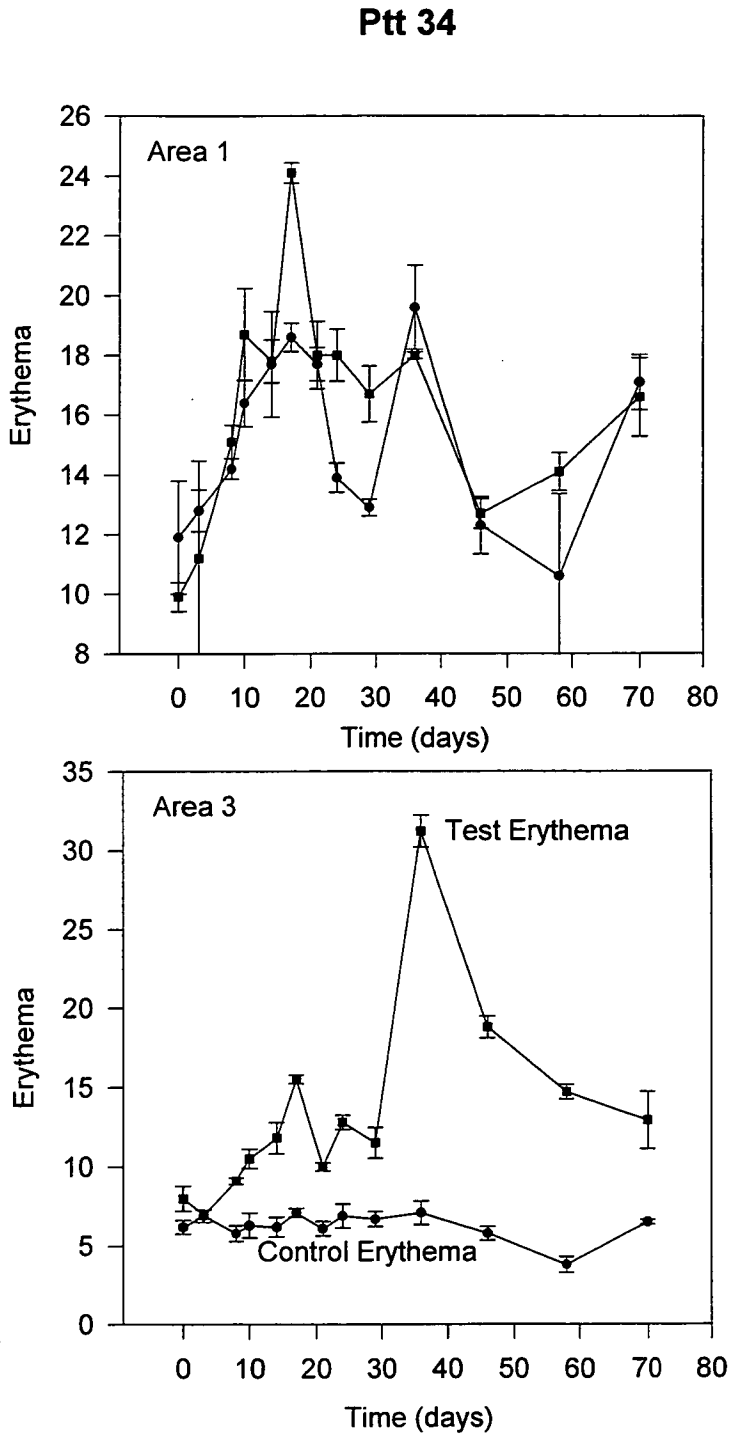


Fig 60 Raw erythema plots of test and control readings over two treated areas in one patient. Area one has no bolus and demonstrates a clear rise in control readings outside the field. Area three has full thickness bolus and demonstrates no significant change in control readings outside the treated field.

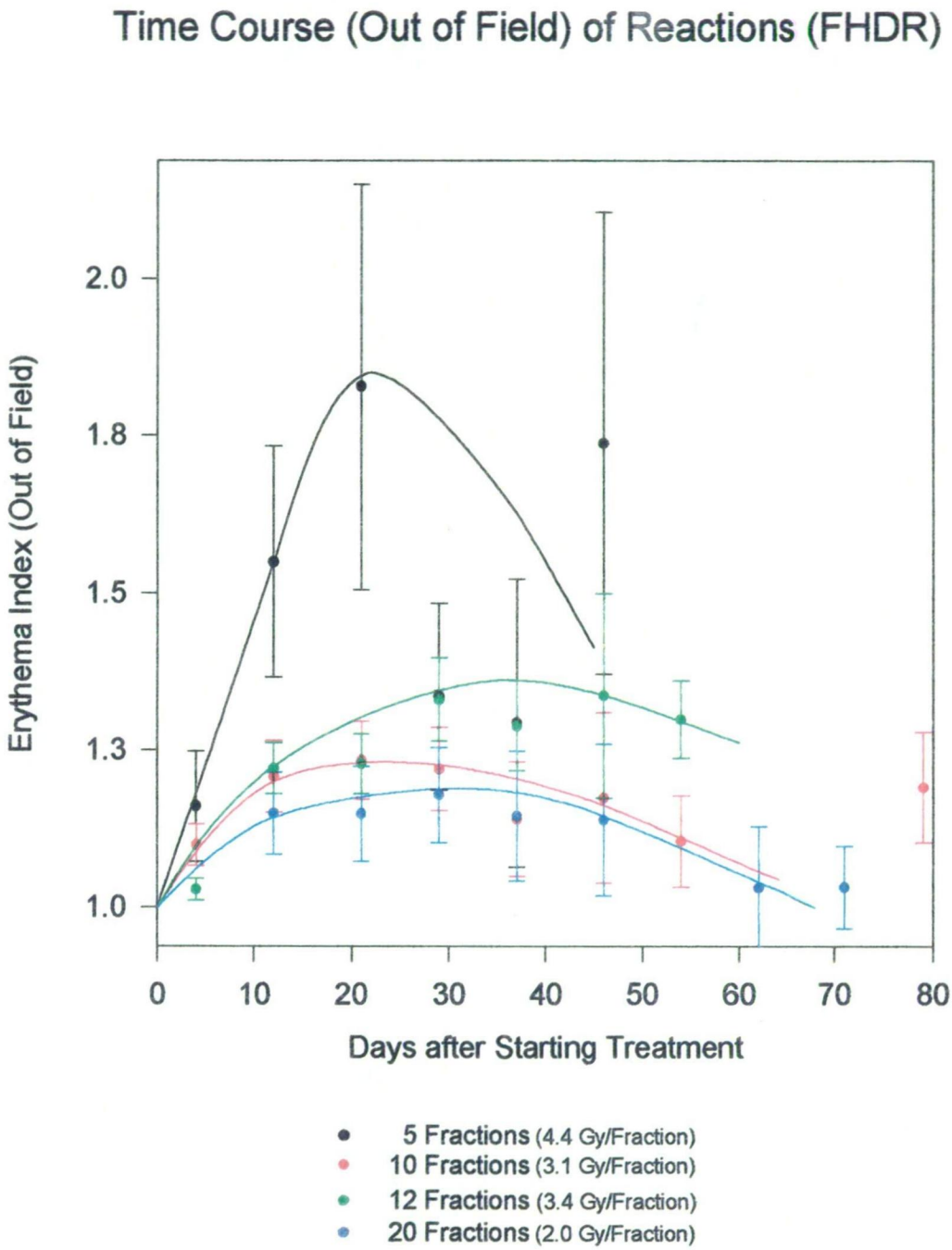


Fig 61 Time Course of Control Erythema readings (Out of Field) for the FHDR group. Curve fits by eye. Error bars: +/- standard error..

14 EFFECTS OF AGE, SEX, SITE AND SUN-EXPOSURE (FHDR)

In general terms, identical trends were noted with respect to both initial erythema and peak erythema for the FHDR data set in comparison to the FLDR patients. Multi-variate linear regression analysis demonstrated that the initial erythema reading was strongly dependant on treatment site ($p<0.0001$), with sites in the lower half of the body displaying both lower initial erythema readings and higher peak erythema index readings relative to sites in the superior half of the body. Again age was found to correlate **negatively** with peak erythema index. ($p=0.0028$) Similar trends were seen with the FHDR patients in terms of the effects of sex and previous ultraviolet exposure on both the initial erythema and peak erythema index however, these co-variates failed to reach significance on multi-variate linear regression analysis. The most powerful predictors of peak erythema index proved to be the treatment dose (ERD) ($p<0.0001$) and the control value, both of which were positively correlated with peak erythema index. Field size (partial area) correlated negatively with peak erythema index. ($p=0.015$) Hair colour, tanning history and melanin content failed to reach significance. Out-of-field peak erythema indices displayed similar trends, however only age reached significance.

15 TLD DOSIMETRY RESULTS (FHDR)

Figure 62 displays the calculated dose versus measured TLD dose for the FHDR patients. This plot displays a small (1-3%) reduction in measured dose over predicted.

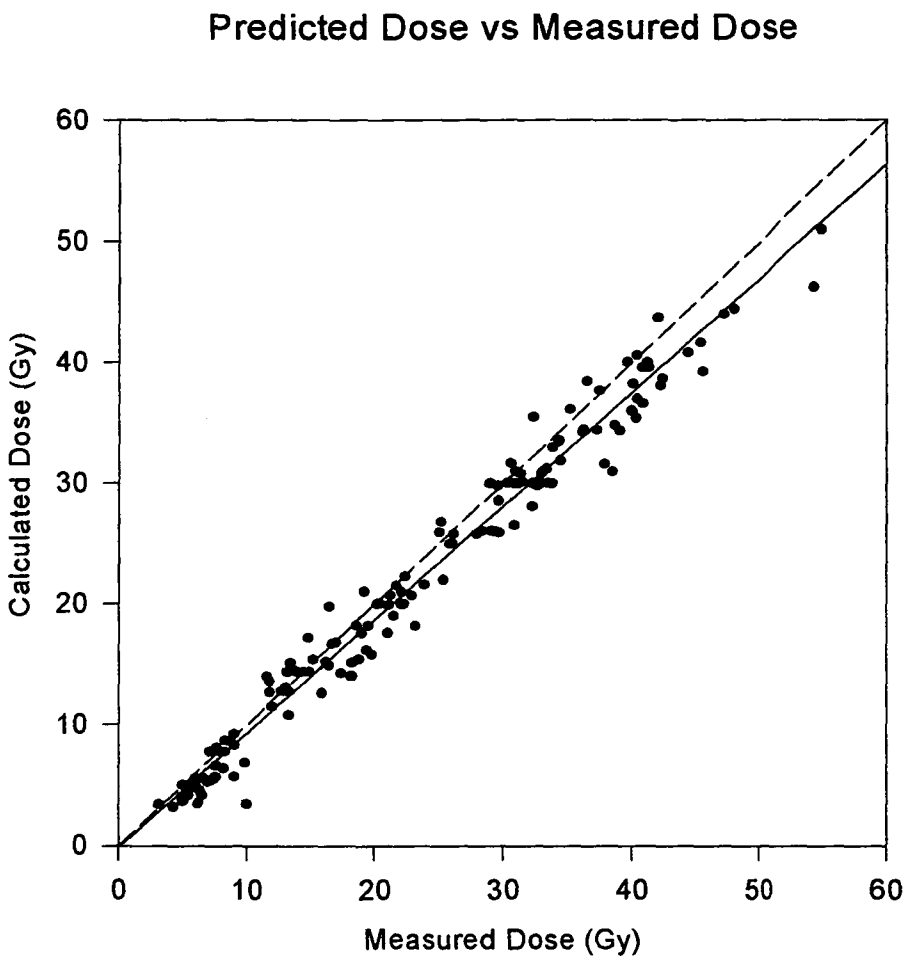


Fig 62 Calculated skin doses versus TLD measured doses for FHDR patients (n=158 measurement areas). Dashed line represents line of unity and the solid line represents the best linear fit to the data.

16 DOSE RESPONSE PARAMETERS (FHDR)

This derivation of meaningful dose response data from this group of patients proved more difficult in comparison to the FLDR patients. Duration parameters, rate of development of erythema and integral measures failed to provide meaningful dose response relationships and the peak erythema index proved to be the only reliable parameter. Figure 63 displays the raw dose response relationships for the 5, 10, 12, 20 and 32 fraction groups. Figure 64 displays the same data, binned for dose.

Figure 65 displays the relationship of the peak erythema index to the rate of development of erythema and in common with the FLDR data set, there is a strong linear correlation, particularly at lower rates of development of erythema. There is a trend to higher peak erythema indices for any given rate of development of erythema for the more prolonged fractionation groups (20 and 32 fractions) versus the lower fraction numbers.

Figure 66 displays the logit fits for quantal dose responses for the FHDR patients using a peak erythema index value greater than 1.8 as the cut-off point for response. This figure clearly demonstrates the more prolonged hyperfractionated regimes (20 and 32 fractions) shifted to the **left** (instead of the right) of the 5, 10 and 12 fraction data. No fit is applied through the 32 fraction data due to the uniformity of dose/fraction.

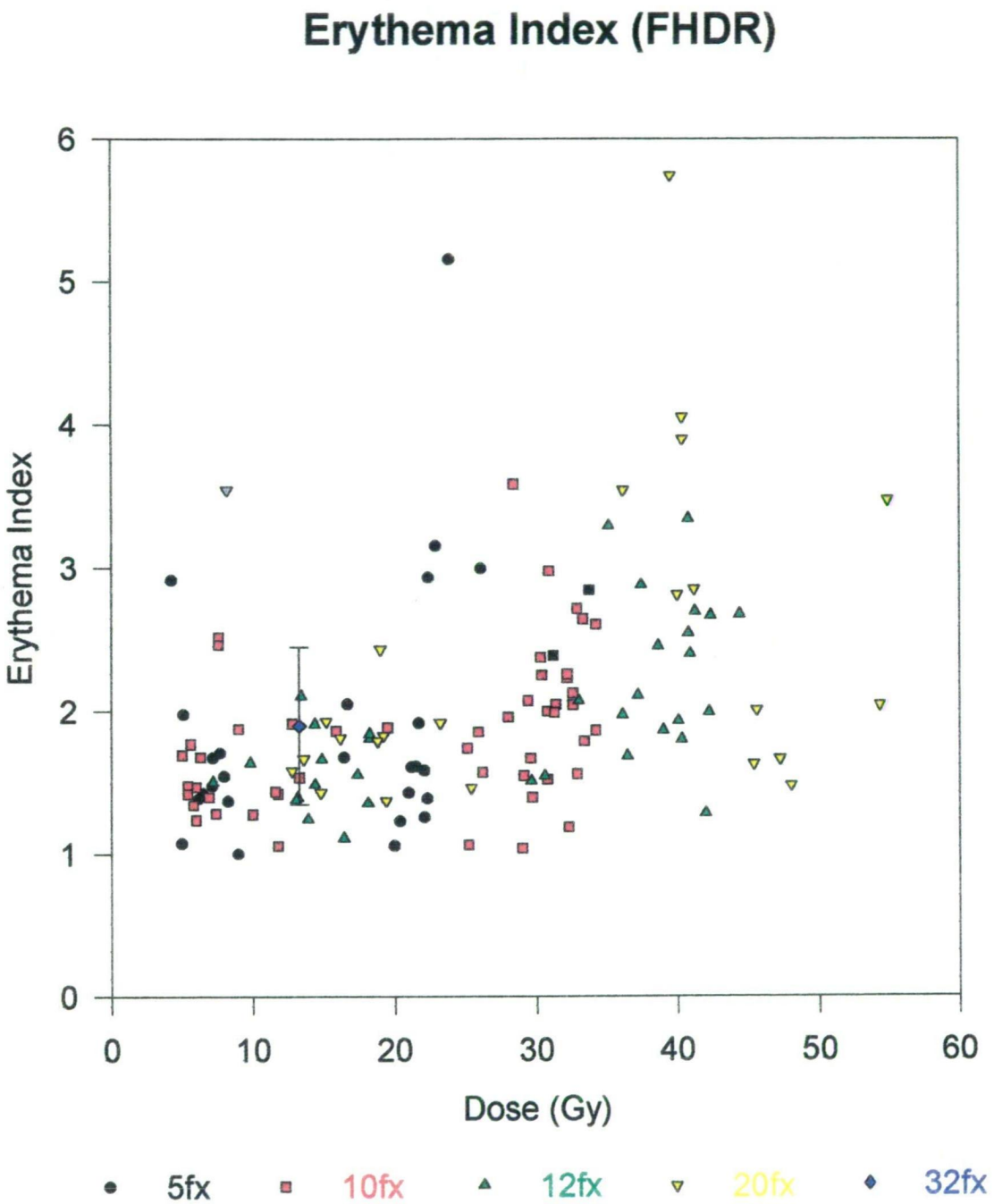


Fig 63 Peak Erythema Index FHDR patients.

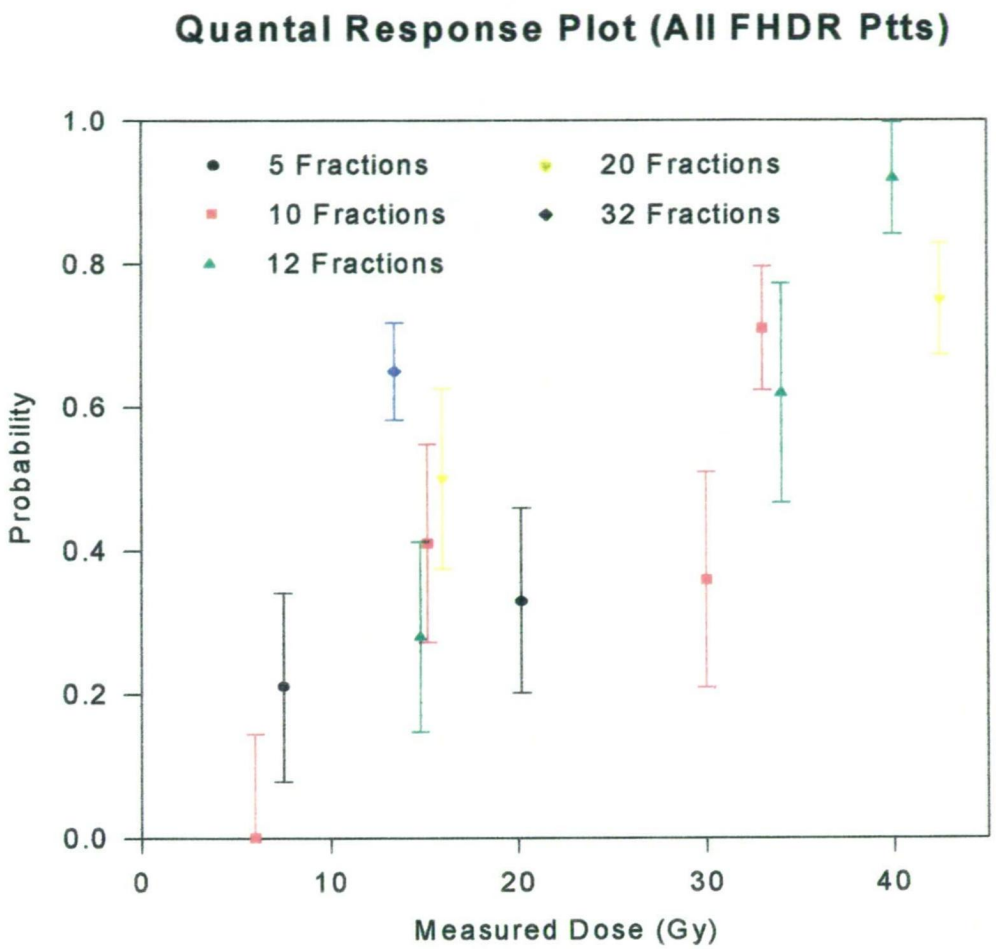


Fig 64 Binned quantal dose response for peak erythema index >1.8 (Error bars +/- one standard error)

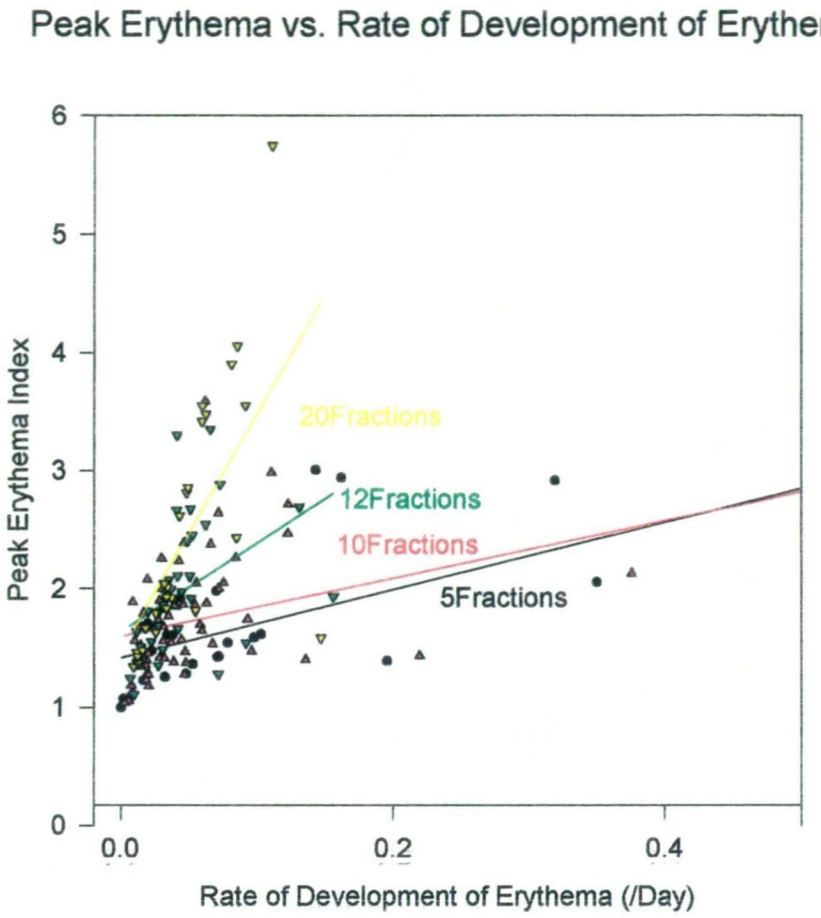


Fig 65 Peak erythema index for FHDR patients versus rate of development of erythema for each of the fractionation groups (5, 10, 12, 20). Each data set has been fitted with a first order polynomial for demonstrative purposes only.(32 fraction data superimposable on 20 fraction data.)

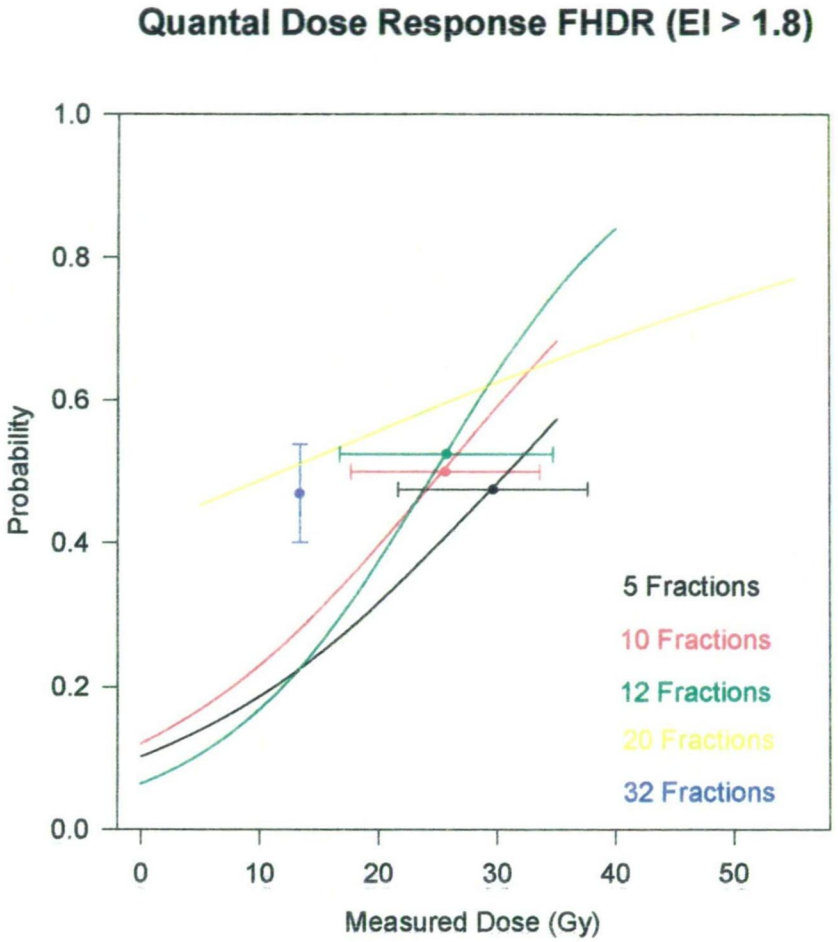


Fig 66 Logit fits for quantal response data for the FHDR patients (EI>1.8). 95% confidence limits displayed for the 5, 10 and 12 fraction data. Confidence limits for the 20 fraction data extend outside the graph.

SECTION D FLDR AND FHDR ANALYSIS

17 COMPARABILITY OF GROUPS

Table 13 displays the make-up of the FLDR, FHDR patients with respect to the previously identified important physiological co-variates (sex, sun-exposure and site). Chi-squared analysis reveals significant biases in distribution of treatment site for each of the groups. This is principally manifest with respect to the head and neck site, with the inclusion of a large group of patients irradiated for head and neck cancer with parallel opposed portals. A similar check of imbalances between co-variates was performed for the FHDR group. Table 14 displays the distribution of the same physiological co-variates amongst each of the fractionation groups. Although statistically not significant on this occasion, there remain some potential imbalances with respect to treatment site.

Table 13

		Study			
		FLDR		FHDR	
Sex p=0.64	Male	73	(68%)	58	(73%)
	Female	34	(32%)	22	(28%)
Sun-exposure p=0.35	No	33	(31%)	17	(23%)
	Yes	74	(69%)	56	(77%)
Site p<0.001	1 Head & Neck	59	(55%)	10	(13%)
	2 Chest	17	(16%)	35	(44%)
	3 Abdomen	9	(8%)	31	(39%)
	4 Hip & other	8	(21%)	4	(5%)

Table 13 Absolute and percentage breakdown of FLDR and FHDR patient groups with respect to sex, previous sun-exposure and site groupings. p values refer to Chi-squared analysis.

Table 14

		Number of Fractions			
		5	10	12	20
Sex p=0.58	Male	9 (60%)	25 (76%)	11 (79%)	9 (64%)
	Female	6 (40%)	8 (24%)	3 (21%)	5 (36%)
Sun-exposure p=0.3	No	3 (20%)	3 (12%)	5 (29%)	4 (28%)
	Yes	12 (80%)	23 (88%)	9 (71%)	10 (72%)
Site p=0.09	1 Head & Neck	0	8 (24%)	0	1 (7%)
	2 Chest	7 (47%)	11 (33%)	9 (64%)	8 (57%)
	3 Abdomen	7 (47%)	14 (42%)	5 (36%)	5 (36%)
	4 Hip & Other	1 (6%)	0	0	0

Table 14 Absolute and percentage breakdown of fractionation groups in FHDR study with respect to sex, previous sun-exposure and site groupings. p values refer to Chi-squared analysis.

17 INDIRECT ANALYSIS

Figure 67 displays the F_e plot for the pooled FLDR data and individual fractionation groups for the FHDR patients. ED_{50} values are derived from the logit fits for the quantal response data (peak erythema index>1.8). If a linear fit is applied to the 5, 10 and 12 fraction and FLDR data then an α/β ratio in the range of 4-10 is obtained. However, from the inset figure, if one assumes the linear quadratic predictions are valid then this implies a short t½ value of 0.05-0.1 hours. The major difficulty arises from the 20 and 32 fraction data which clearly is not fitted by a positively sloped linear function through the remaining data point. If anything the 20 and 32 fraction data suggest a **negative** α/β ratio. The poor fit of the FHDR data to the linear quadratic model is further confirmed in a de Boer and Tucker plot. (figures 68 and 69) Neither of these plots approximate a linear function.

The entire data set may also be viewed on a "survival curve" plot (figures 70 - 72). Figure 70 displays binned FHDR and FLDR data with predicted LQ curves (for high dose rate only) superimposed. It is readily apparent that the FHDR data does not fit well on any of the LQ predicted curves in the low dose per fraction region.

The best R^2 value for a linear quadratic fit is 0.21. Fits using a third order polynomial (with a negative coefficient on the quadratic term) produces the "S-shaped" curve with an R^2 of 0.89.

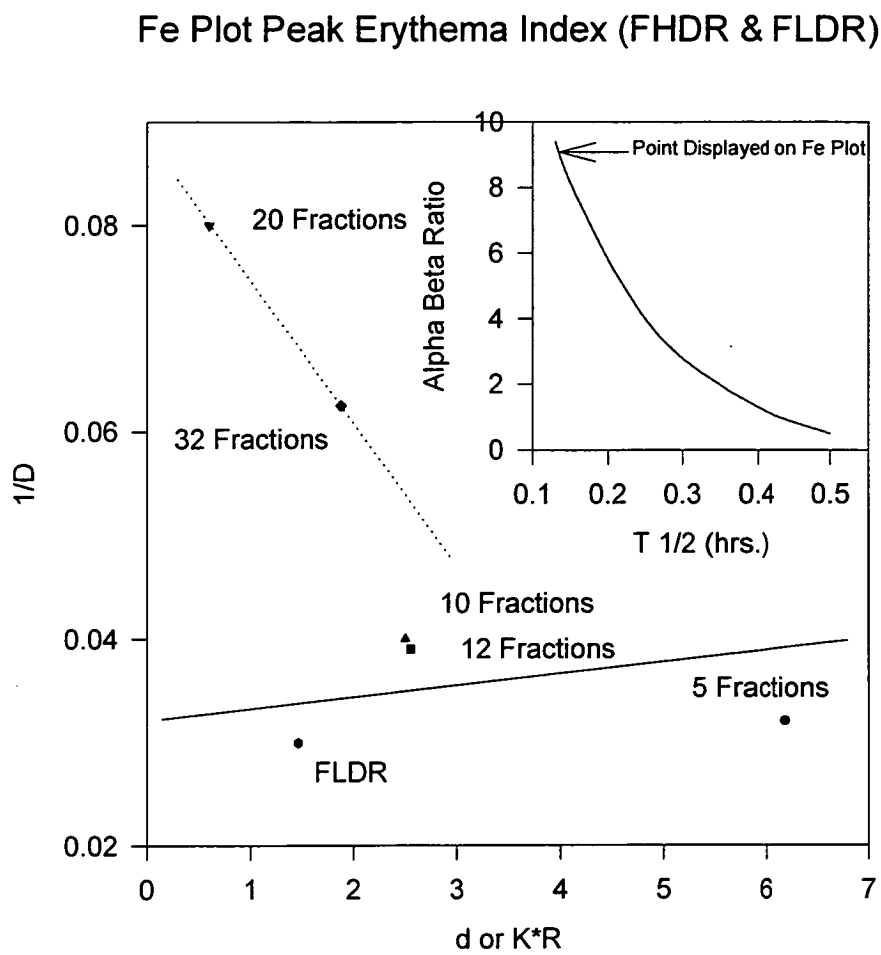


Fig 67 Modified reciprocal dose plot for all FLDR (pooled) and FHDR patients. Inset refers to the relationship of the ordinate intercept (α/β) to the t½. (see Section 8; Introduction) Solid line refers to a fit through 5, 10, 12 fraction and FLDR data. Dotted line represents a fit through 20 and 32 fraction data.

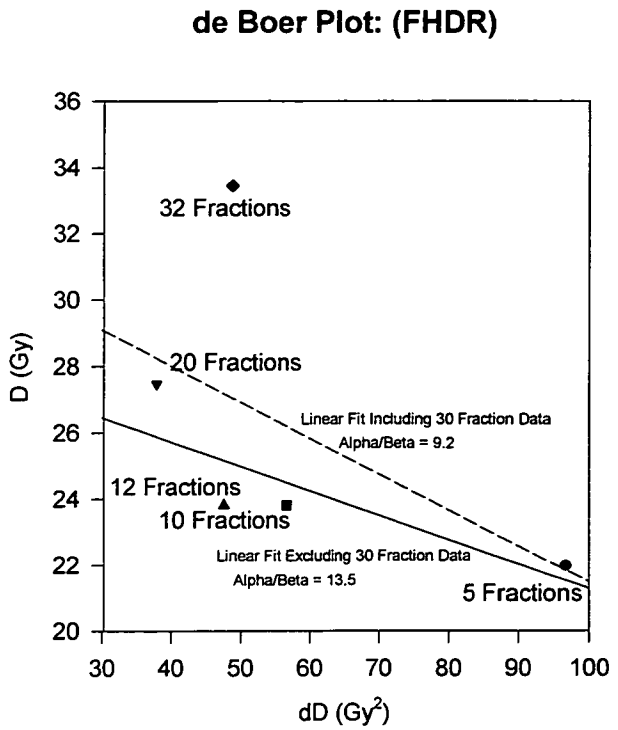


Fig 68 de Boer plot of FHDR data (peak erythema index > 1.8)

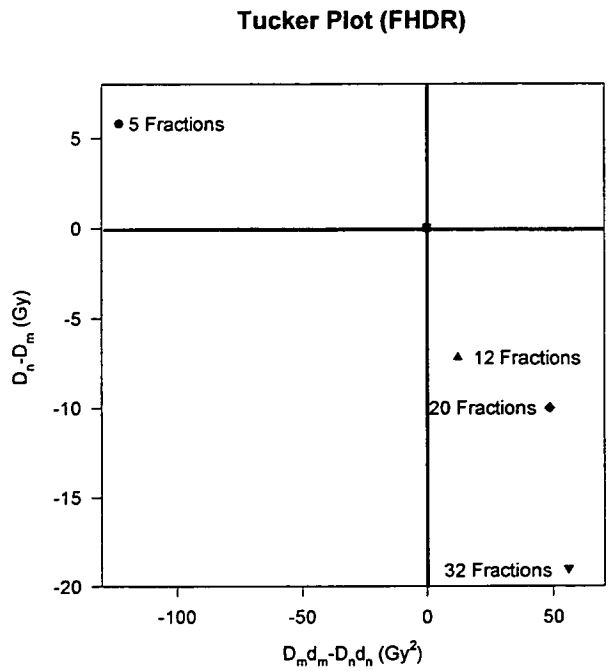


Fig 69 Tucker plot for FHDR data (peak erythema index >1.8) Reference fractionation group taken as 10 fractions.

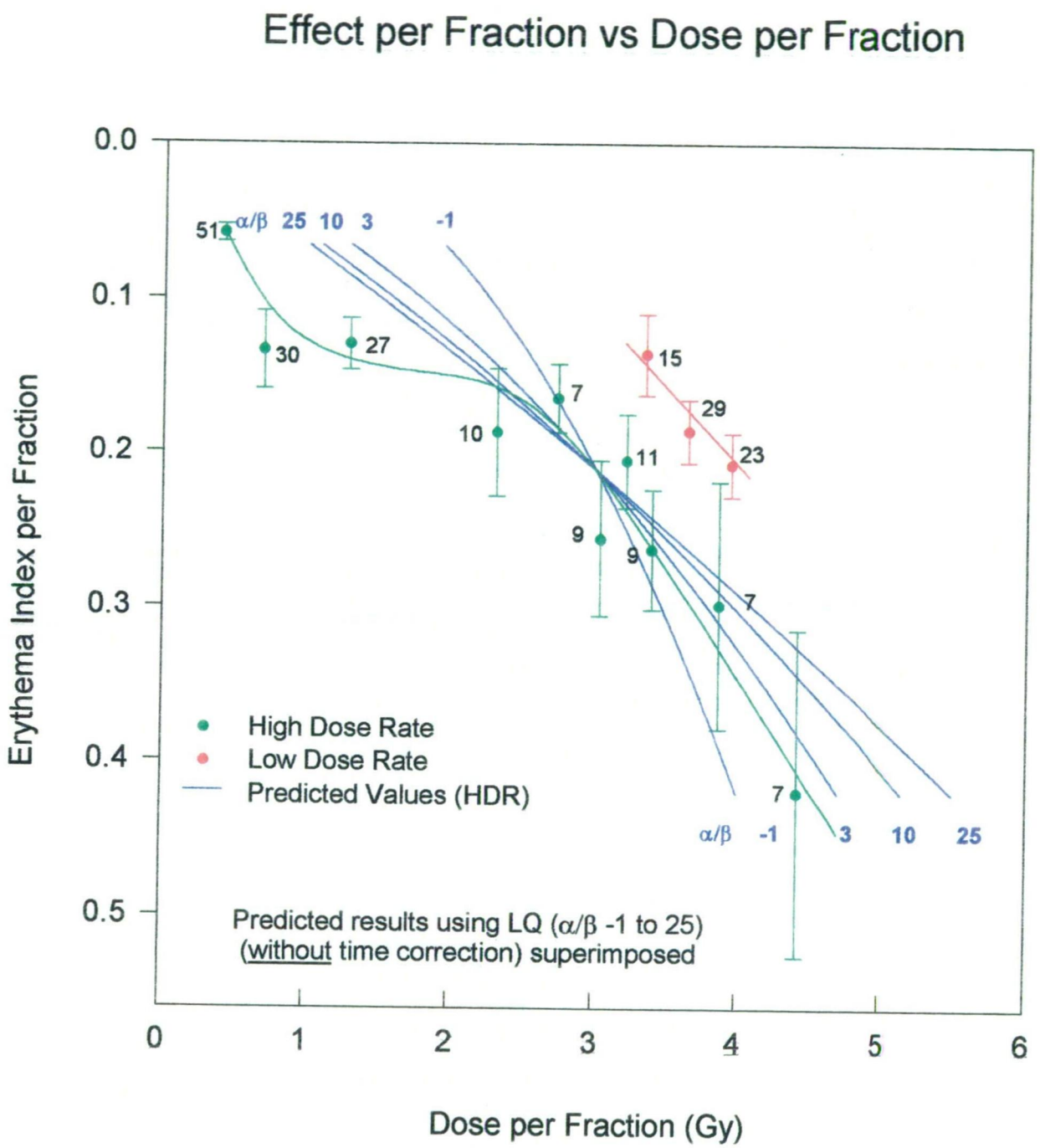


Fig 70 Plot of peak erythema index per fraction vs dose per fraction for all FHDR and FLDR patients (excluding H & N ptts). Data has been binned and patient numbers are given for each point. Error bars: +/- 1 standard error. Predicted high dose rate linear-quadratic curves are superimposed for comparison.

Figure 71 displays the same data, however, on this occasion with linear-quadratic predictions modified for the effective time (assuming a potential doubling time of 5 days and a "kick-off" time of 10 days)(no time correction for FLDR data). In this instance, again the fit of the FHDR data to LQ prediction is poor, particularly in the low dose per fraction region.

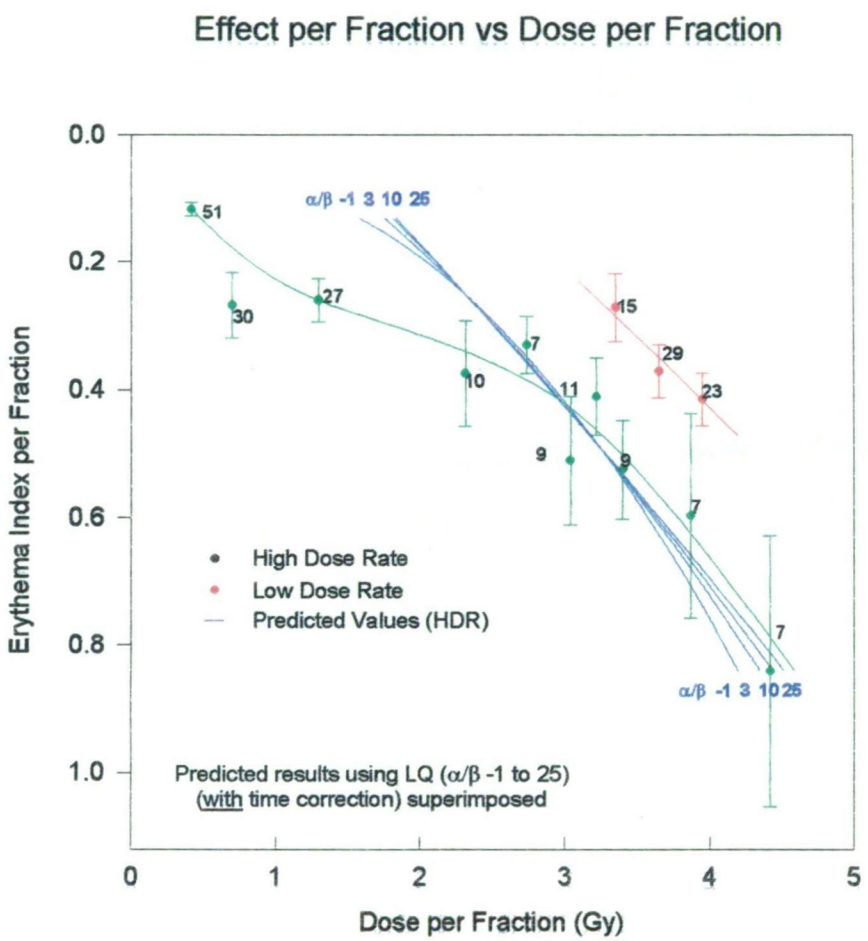


Fig 71 Plot of peak erythema index per fraction vs dose per fraction for all FHDR and FLDR patients (excluding H & N) with time correction. Data has been binned and patient numbers are given for each point. Error bars: +/- 1 standard error. Predicted high dose rate linear-quadratic curves are superimposed for comparison.

Other approaches were sought to explain this poor linear-quadratic fit. Figure 72 displays the results of a "survival" curve derived from the absolute change in erythema units (peak values) rather than a normalised approach. (This transform is given in Results, Section 4: Transform 3) Again it is clear that there is a less marked but evident S-shape to the dose response function. (R^2 for Linear Quadratic fit; 022. R^2 for cubic fit; 0.78)

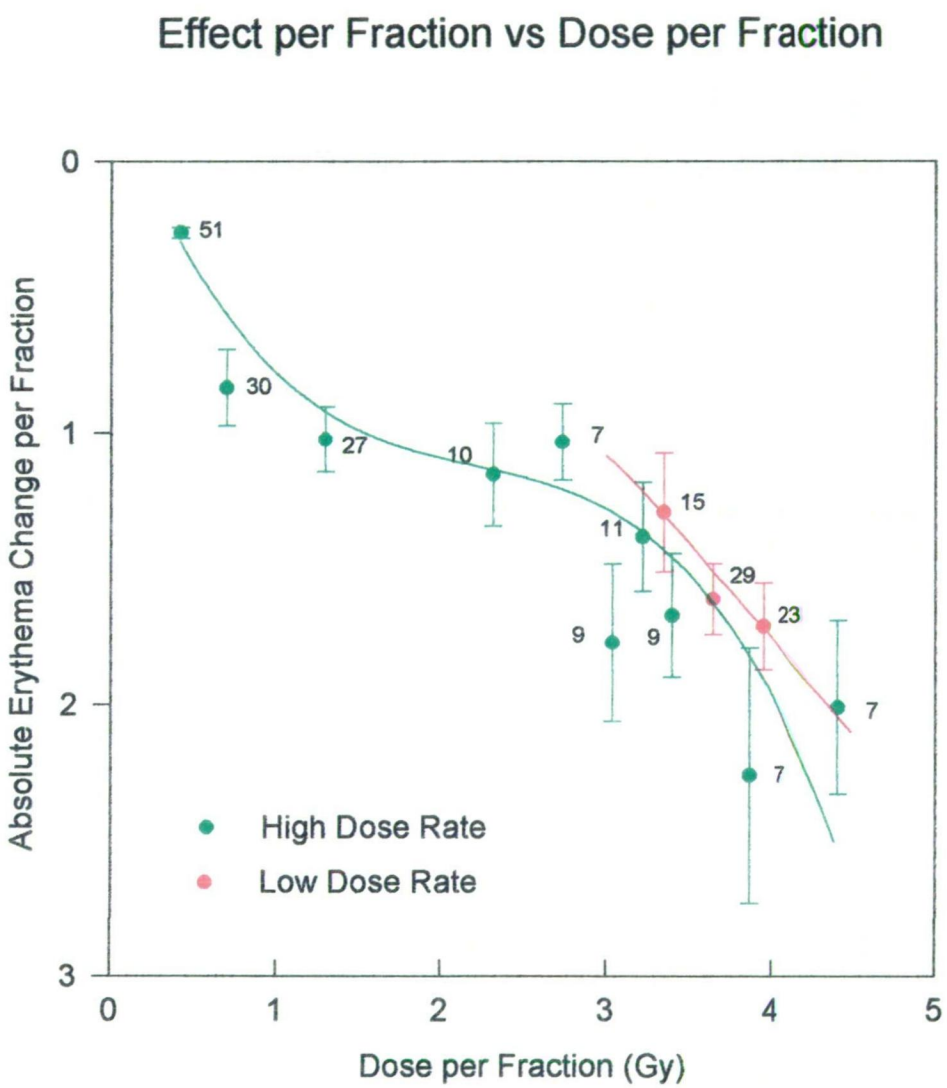


Fig 72 Absolute change in erythema per fraction vs dose per fraction for all FHDR and FLDR data. Data has been binned with patient numbers given at each point. Error bars: +/- 1 standard error.

In light of the out-of-field effects noted particularly in the FHDR data set, dose response data comparing patients having one or multiple test sits was performed (this was on the assumption that adjacent bolused areas may have contributed to the radiation response in an adjacent unbolused area).(Figure 73) Although there may be some tendency for the single areas to show less effect at the same dose level than the bolused areas, these data points (along with the prostate data) still do not fit the Linear Quadratic function well. (R^2 = 0.31)

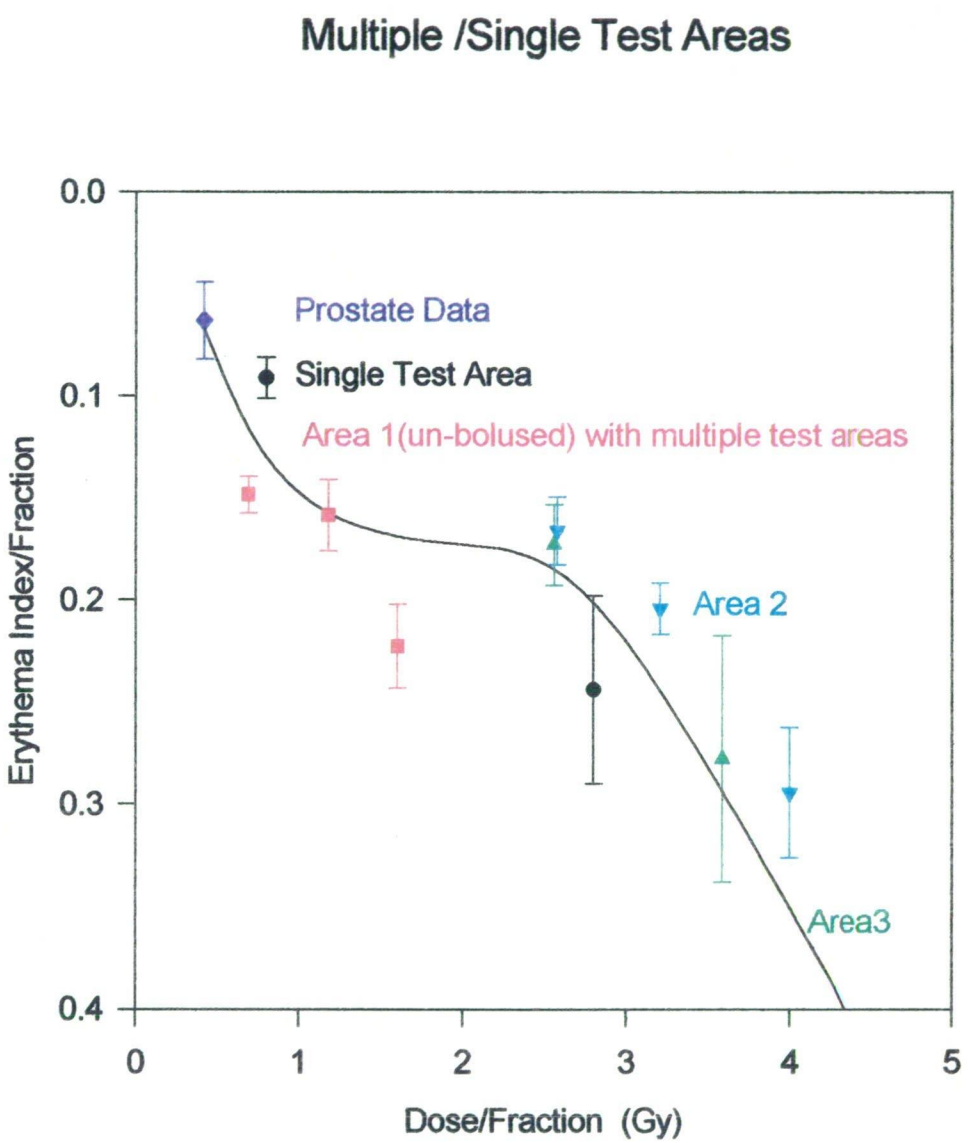


Fig 73 Peak erythema index per fraction vs dose per fraction for FHDR patients having one or more test areas. Error bars: ±1 standard error.

18 DIRECT ANALYSIS

Direct analysis using the ABest program for all patient in the FHDR, FLDR study groups produced negative α values and therefore a negative α/β ratio of -0.9 and a t½ value 0.23 hours. Positive α/β ratios could only be obtained by restricting the analysis to dose rates above 0.8 Gy/hr (FLDR) and exclusion of the 20 and 32 fraction data (FHDR) or exclusion of all the low dose per fraction data (FLDR). Subset analysis of the FHDR, FLDR groups to confine the FHDR data to the 10 fraction group only and the FHDR data to single field data only (excluding head and neck sites, in view of the possible effect of different dose rates to the skin during each treatment) gave an α/β ratio of 6.9 and t½ of 0.06 hours. These later figures correspond closely to the direct analytic technique results given in figure 67. Similarly, ABest analysis of the high dose per fraction data gives an α/β ratio of 7.1 and t½ of 0.08 hours. Table 15 displays the α/β ratios, t½ values and their confidence limits for the entire FHDR, FLDR groups and subset analyses.

Table 15

Groups		Poisson		Logistic	
FHDR	FLDR	α/β (Gy) range	t½ (hrs) range	α/β (Gy) range	t½ (hrs) range
5,10,12,20 ,32 fx	all	-0.9 (-2.2 - 2.1)	0.2 (0.11- 1.4)	-0.6 (-2.1- 2.9)	0.2 (0.11- 1.97)
5,10,12,20 high d/fx	all	7.1 (-3.0- 10)	0.08 (-0.14-3.2)	NC	NC
10 fx	skin only	6.9 (NC)	0.06 (-0.19, 0.31)	NC	NC

Table 15 ABest maximum likelihood estimates of α/β and t½ for FHDR and FLDR study groups and subgroups. Confidence limits are indicated in parenthesis where they are able to be calculated.

19 SUMMARY

- Reflectance spectrophotometry provides an accurate and reproducible quantitative measure of baseline human skin erythema. Considerable intra-patient heterogeneity has been demonstrated with both extrinsic and intrinsic physiological factors impacting on initial and x-ray perturbed erythema values. These variable include;

  sex - females showing a lower initial erythema but higher peak erythema index than males.

treatment site - sites in the lower portion of the body having a lower initial erythema but higher peak erythema index.

age - older patients having a higher initial erythema but lower peak erythema index than younger.

sun-exposure - showing identical trends to site, with non sun-exposed sites exhibiting lower initial erythema readings but higher peak erythema indices relative to sun-exposed sites.

melanin content - higher initial melanin content of the skin was associated with a higher initial erythema reading but lower peak erythema index.

- Reflectance spectrophotometry was significantly more sensitive than a semi-quantitative grading scale.

- Reflectance spectrophotometry demonstrated dips (which often extend below base-line values) at approximately 10-14 days in a subset of patients.

- Reflectance spectrophotometry demonstrated measurable reciprocal vicinity effects at approximately 3 cm from the field in a subset of patients.

- Reciprocal vicinity effects were demonstrated predominantly in the FHDR data set, demonstrated similar time coursed to in-field reactions and were most marked in younger patients.

- Dose time plots were best derived using a normalised erythema index approach ($E_i = E_t/E_0$).

- Peak erythema index provided the best measure of radiation dose response.

- Peak erythema index was strongly correlated with the rate of development of erythema.

- Rate of development of erythema was dependent on size of dose per fraction in the FHDR group and there was a suggestion of a similar dependence on dose rate in the FLDR group.

- Inverse fractionation effects were seen in the FHDR data set resulting in a negative α/β ratio for the entire group of patients.

- Subset analysis on patients treated at high doses per fraction suggested an α/β ratio in the vicinity of 7.1 and t½ of 0.08 hrs.

- Direct and indirect analytic techniques demonstrated a poor fit of FHDR data to the linear quadratic function, particularly at low doses per fraction.

- The FHDR data was well fitted by a third order function. (negative quadratic coefficient)

CHAPTER IV DISCUSSION

1 STUDY DESIGN

Any clinical radiobiology project which seeks to accurately define the fractionation sensitivity (or dose rate effect), in a human population would ideally encompass a wide range of doses per fraction (or dose rates) in order to derive a maximal data envelope on which to base direct or indirect analyses. Similarly, a wide spread of inter-fraction intervals in a data set is optimal to allow modelling of inter-fraction repair processes (or intra-fraction for FLDR). A variety of patient, physical, engineering and logistic problems inevitably meant that this study design represented a compromise between these two competing considerations. With regard to dose rate, the 0.8 Gy/hr treatment group represented the only dose rate group which would be considered to be in the low dose rate range, with 3, 4.8 and 8 Gy/hr more conventionally considered to be in the medium dose rate range. Treatment times over 5 hours per day are impractical and the dose rates selected, represented a range over which it was felt that a dose rate effect might be detectable clinically, in acutely responding tissue systems such as skin and mucosa. Similarly, in the FHDR group, it was not possible to design a study which encompassed a range of dose per fraction sizes beyond the range 50 cGy to 550 cGy.

Patients were accrued sequentially onto a central composite design which should minimise selection bias. Differing field arrangements, field sizes and patient anatomy inevitably dictated that precise prospective dosimetry could not be guaranteed. This has produced a relative lack of dose data in the range 2-3.5 Gy per fraction. This may have contributed to some of the difficulty in coherent separation of dose response curves and later LQ analysis, however one major benefit of the clustering of doses under 1 Gy/fraction was that evidence was produced for under-prediction of the LQ model for effects at low doses per fraction .

Quite apart from these physical and dosimetric considerations, previously published differences in skin response to radiation at varying sites would normally dictate that an "ideal" study should confine itself to one skin site. In the context of a medium-sized radiotherapy treatment centre, with study patients treated palliatively, one is faced with a very limited set of choices. Given that accrual to this project required some four years, then selection of an appropriate single site for the FHDR study became an impossibility. (apart from the prostate group) It may also be argued that derivation of parameters for the radio-responsiveness of skin at one site in a select group of patients may have limited clinical applicability to the wider arena of therapeutic radiology where a multiplicity of treatment sites are involved. A similar argument applies for the heterogeneity of important intrinsic and extrinsic physiological co-variates such as sex, age and

previous sun-exposure. Given our time again, we would have prospectively stratified for these variables, however their importance was not appreciated (or published) at the outset of the study. Heterogeneity of the study population with respect to all these factors contributed significantly to pre-treatment and treatment related inter-patient variability and therefore the quality of both the indirect and direct quantal analyses, however on the plus side, did allow for significant new quantitative insights to be gained into the effect of these variables on radiation response.

The matrix of total doses and dose rates chosen for the FLDR study were based on known linear quadratic predictions and measured skin doses therefore tended to cluster reasonably reliably around prescribed doses (prescription point to midline for head and neck patients and to D_{max} for skin patients). This was not the case for the FHDR project where prescription points were commonly at depth for multi-field setups and a much broader spread of measured doses were seen. The other important difference in design between the FHDR and FLDR projects, relates to the common use of multiple measurement points within the one field in one patient in the FHDR project. (75% of patients had more than one measurement point). (see section 1.2 Materials and Methods). This methodology was adopted from the outset of the study as a means of generating a greater number of dose response points. "Out of field" effects of radiation were not appreciated or anticipated at the inception of the study. Similarly, the decision to obtain a control reading 3cm. outside of the field was found in retrospect, to be of no value in terms of serving as a useful control, however the finding that these control points exhibited erythema dose responses which were similar to in-field responses proved equally valuable in its own right.

2 DOSIMETRIC CONSIDERATIONS

The derivation of meaningful dose response curves for a study such as this is critically dependant on reliable skin dosimetry. Although parallel plate ion chamber measurement can be performed in the in-vivo situation, (Russell et al. 1994) TLD's represent a more practical solution to the majority of radiotherapeutic applications. The TLD results in this study are within the commonly accepted range (3-5%) of variation in results using this technique. In both the FLDR and the FHDR projects, they correlate well with predicted dose. In both studies there is a marginal negative difference in the measured doses versus predicted, particularly in the higher dose region (35 Gy +). This may reflect a small under-adjustment for supra-linearity of TLD response. Alternatively, this observation could be due to the reduction in side-scatter and back-scatter occurring with irregular surfaces or non-orthogonal patient set-ups, when compared to the original phantom measurements, in which the beam incidence is always orthogonal and full back scatter conditions exist. In order to assess the effect of this possible under-prediction of supra-linearity, the indirect analysis of the FLDR

and FHDR data was repeated using a +3% supra linearity dose correction at 40 Gy. This adjustment made no material difference to the dose response curves or F_e plots.

Patients treated in the FLDR study to the head and neck region with parallel opposed fields, in effect, received each fraction of FLDR at two different dose rates, differing by approximately 15%. Given that the iso-effect curves in the dose rate region under consideration are relatively flat, then an average of these two dose rates should provide reasonable equivalence with the two actual dose rates delivered. Similarly, for multi-field patients in the FHDR study, the measurement point may have received two or more, nearly instantaneous, contributions from entry, exit and possibly lateral fields over a period of 5-10 minutes. Again for instantaneous dose rates in the range 100-500 cGy/min and an overall fraction delivery time which is considerably less than the expected repair half times, then one would not expect any significant biological difference than the same total dose delivered in one continuous FHDR fraction to a single field.

Finally, the other major potential physical difference between the study groups relates to average beam energy. For Caesium 137 this is 660 kv and for 6 MV photons is 3.8 MV. Traditionally the relative biological effectiveness (RBE) of 6 MV and Cobalt 60 have been assumed to be unity and likewise most workers have assumed the RBE of caesium 137 to be equivalent to that of cobalt 60 (Millar and Canney 1993).

3 REFLECTANCE SPECTROPHOTOMETRY UNIT

3.1 The Physical Characteristics

We found the unit employed in this study to be a robust and reliable instrument, capable of highly reproducible data in terms of haemoglobin index and to a lesser extent, oxygenation and melanin index on artificial surfaces. The measurement technique proved to be simple and independent of observer. For baseline readings in unperturbed skin, the haemoglobin index showed further variation over and above that recorded for artificial surfaces, however, was still relatively low with an intra-patient coefficient of variation of 11.9 - 12%. Consistent re-measurement of the same site of skin proved readily possible, as did the elimination of the effect of skin blanching due to excessive pressure on the skin by the measurement head.

The unit employed in this study is significantly different in terms of methodology to derive a haemoglobin index, to that reported in other studies. (Nias 1963; Turesson and Notter 1976; Russell et al. 1994) Previously utilised reflectance units assess reflectance at single wavelengths,

corresponding to oxy or de-oxyhaemoglobin peak absorbancies. Because the total reflectance value for haemoglobin is dependent on the amount of de-oxyhaemoglobin present, the unit employed in this study derives a haemoglobin index via the difference in LIR at 3 isobestic points. (wavelengths at which the LIR values of oxy and de-oxy haemoglobin are equal), which is a function of the haemoglobin content of the skin, independent of de-oxyhaemoglobin.

Other workers have noted the dependence of measured erythema on melanin content (Russell et al 1994; Turesson and Notter 1976) however, the present instrument quantitatively accounts for the presence of melanin in varying concentrations by a subtractive correction based on a "pigmentation index" derived from the slope of the melanin absorbence spectrum at several different wavelengths. Oxygenation index as measured by the reflectance unit had large intra-patient variation and was found to be of extremely limited value in terms of both a baseline measure and in response to radiation. This parameter is more closely dependent on blood flow rates in the skin and is therefore more analogous to Laser-Doppler measurements, which have also tended to display higher variability rates.(personal communication. W. Dörr)

3.2 "Artificial Erythema Study"

In common with Dawson et al. 1980, we demonstrated a strong linear dependence of haemoglobin index values on the concentration of a suitable flesh coloured pink powder used for construction of prostheses. The concentrations of pigment chosen for the tiles were deliberately selected to correspond to realistic shades of erythema seen in the clinical situation. The highest concentrations of pigment represented various significant reaction grades in an artificial sense and were associated with a supra-linear response from the reflectance unit. In both the FLDR and the FHDR studies, erythema values rarely exceeded 50 and most peak reactions were more commonly in the range of 20 - 40, where supra-linearity. was not evident. Given the lack of a translucent reflective surface (forming the wall of a reflectance cell) in the tile experiment, then application of these results to the human situation must be cautious. Dawson and Feather 1980 and 1989, have provided additional evidence for a linear dependence of the haemoglobin index on the concentration of haemoglobin by construction an artificial reflectance cell from a matt slide, red cell suspension and cuvette, in order to simulate the in-vivo situation. For both whole red cells and haemolysed cells, they demonstrated a clear linear response over a three fold change in concentration of red cells. There is good evidence from these and other studies that this reflectance unit reliably measures the "pinkness" of human skin but just what relationship this bears to micro-vascular anatomy and physiology awaits further study.

4 CONTROL STUDIES

4.1 Normal Individual

Erythema readings performed daily over eight abdominal measurement points in a normal female initially suggested some cyclical variation with a phase of some 3 - 4 days. This was not confirmed with a smoothing algorithm and control theory suggested that no systematic variations were occurring in the data set as a whole. Significant geographical variation in erythema readings was noted in a superior to inferior direction in common with Turesson and colleagues 1976. They noted slightly decreased erythema values in the lower portion of parasternal fields and attributed this to a higher skin sympathetic tone in the region. Interestingly this study also demonstrated a significant left to right difference in erythema readings. Neither of these trends could be attributed to a systematic drift in the reflectance unit associated with warm-up. This lateralised difference must therefore be either due to chance or lateralised physiological or neuro- physiological processes. Small left to right differences in pre-treatment erythema were noted by Turesson & Notter 1984, (Dose Rate III) possibly as a result of the previous mastectomy.

4.2 Cancer Control Patients

The motivation for the cancer control study on erythema measurements was two fold; firstly to detect any systematic trend that may be occurring in erythema measurements related to factors other than the radiotherapy delivery to the treated site itself. This included possible non-specific environmental and or emotional effects, possible effects due to the malignancy itself and abscopal effects due to the delivery of radiotherapy at a site distant from the measurement site. Patients selected for this study were of similar age and were matched for sex and history of ultraviolet history to the measurement site. They were however, generally treated with radical intent, often in adjuvant settings and therefore represented a different biological subset, in terms of their malignancy, to those patients undergoing treatment in the FLDR and FHDR studies. Not withstanding, the cancer control studies broadly confirmed the same findings as those demonstrated in the normal individual. That is, there was usually a clear absence of significant temporal trends and typical intra-patient variance of around 10%. There were however, two control patients who showed a significant upward and downward trend during their course of radiotherapy which proved impossible to clearly explain.

Secondly, this study failed to demonstrate any meaningful relationship between erythema index and the physiological parameters of blood pressure,

pulse rate, respiratory rate and skin temperature. This finding is probably related to the relatively steady state of physiology under which the patients were measured. Feather and Dawson 1989 have previously demonstrated reproducible changes in the haemoglobin index associated with positioning of the forearm at different heights relative to the heart. Raising the forearm well above the level of the heart produced a clear drop in haemoglobin index and a rise in oxygenation index. Kollias and Baquer 1988 demonstrated a clear relationship between measured erythema (reflectance spectrophotometry) on the forearm of volunteers related to inflatable cuff pressure of a proximal arm pressure cuff. We did observe a significant negative correlation of the oxygenation index with skin temperature. This may reflect reduced dermal oxygen transfer occurring with altered microvascular blood flow (e.g. reduced A-V shunting). Similarly, there was clear correlation of the oxygenation index with the erythema index in the cancer control patients. This presumably reflects enhanced oxygen transfer and blood flow rates associated with greater vasodilatation.

4.3 Pre-treatment Inter- and Intra-patient Variation

Of more interest in terms of deriving radiobiological data, is the observation that pre-treatment erythema values had inter-patient coefficients of variation of the order of 40%. This immediately implies that there are at least several possible pre-treatment variables (intrinsic or extrinsic) which impact on baseline values (quite apart from x-ray perturbed values). It was readily apparent from both uni-variate, multi-variate and generalised linear regression analysis that the predominant factors impacting on this inter-patient heterogeneity were sex, previous significant sun-exposure to the measurement site and the location of the measurement site itself. Depending on the methodology chosen and the exact number of covariates employed, either previous sun-exposure or treatment site proved significant on multi-variate linear regression analysis and given their intimate inter-dependence, it is impossible to meaningfully state that either are independently significant. Forward stepwise regression techniques and best sub-set analyses allow selection of the optimum combination of co-variates and did suggest that site was the stronger of the two. Thus, sun-exposed sites in males were found to have the highest baseline erythema readings and in turn the highest intra-patient coefficient of variation. At the other end of the scale, non-exposed females tended to have considerably lower initial erythema readings with a lower intra-patient coefficient of variation. Identical patterns of initial erythema variability were seen in both the cancer control group and the FHDR group.

These findings almost certainly reflect intrinsic micro-vascular anatomy associated with previous UV exposure and/or site with probable additional effects associated with sex, which may be intrinsic (e.g. hormonal) or lifestyle related. High intra-patient coefficients of variation in previously sun-exposed sites may reflect impaired micro-vascular homeostatic mechanisms. A further possible explanation for the observed baseline erythema difference between the sexes, is the known difference in serum haemoglobin concentration.

A consistent but numerically weak negative correlation existed in both the data sets, with respect to age and baseline erythema. Kelly et al. (unpublished data 1994) have demonstrated, using video capillaroscopy and fluorescein angiography together with Laser Doppler measurements, that there was a significant reduction in dermal papillary loops (nutritional exchange vessels) in older skin compared to young subjects. This reduction was found in both the forehead and forearm suggesting that it may result from both chronological and photo-ageing. Sub papillary plexus vessels however, were not significantly diminished in the elderly, and they noted gross dilatation, tortuosity and disorganisation of these vessels. It may be that changes such as this group observed are responsible for the finding of a negative correlation with age.

Anderson and Bjerring (1990) demonstrated significant regional variation in erythema measured by reflectance spectrophotometry in six volunteers (5 males, 1 female). The results closely mirrored the findings of this study. For example, an erythema reading of 5.7 was found for the thigh and on the cheek 59.5. A gradient of measures between these two sites corresponded closely to the measured values of this study. They reported no significant differences between identical sites on the left and right side of the body. Their measurements also demonstrated a greater coefficient of variance for sites in the superior portion of the body which are more commonly sun-exposed. The cancer control patients demonstrated an intra-patient variability of 12% for this reflectance unit, which compares with a value of 31% reported by Russell et al. 1994. In their study a tri-stimulus colorimeter was employed at a single site (post-mastectomy chest wall). This unit does not measure haemoglobin concentration per se, rather it assesses the colorimetric contribution of any red pigment in the site under consideration. Their higher degree of intra-patient variability may reflect technical or possibly post-operative factors.

It therefore seems likely, based on ours and others observations of unperturbed erythema values, that a variety of intrinsic physiological and extrinsic factors (principally previous significant UV erythema), contribute not only to the baseline level of erythema at any given site, but to its degree of variation at an inter and intra- patient level.

4.4 Defining the Erythema Index

In common with Turesson and co-workers (1976), we found the most valuable measure of erythema change to be an erythema index as defined by relating any given value at time t to the initial erythema readings.

$$E_I = \frac{E_t}{E_0}$$

This is essentially a simple normalisation technique which will correct for baseline inter-patient variation. It has the disadvantage of relatively inflating erythema increases with treatment for sites with a low initial erythema value over sites with a higher initial value. This of course, assumes that the rise in erythema with treatment is independent of the initial starting value.(ie depends only on time/dose factors) Several key analyses were reproduced using the absolute change in erythema (transform 3; section 3.2) rather than the normalised approach. For multi-variate analysis of peak response, nearly identical trends were demonstrated for age, sex, site etc., however the dose response plots were inferior to transform 2. The F_e plot derived from transform 3 produced a somewhat lesser degree of inverse fractionation for the 20 and 30 fraction data, but still not sufficient to suggest a linear fit. This is seen in the organ based "survival" plot (figure 72) which displays a poor fit to the linear quadratic model at low doses per fraction (adjusted R^2=0.21).

Re-analysis of the data using Turesson's definition of erythema index;

$$E_I = \frac{E_t - E_0}{E_0} \times 100$$

produces identical dose response plots to the previous expression. This is not surprising given the mathematical similarity of the two expressions.

4.5 Usefulness of Control Erythema Readings

In view of previously published observations and our own expectations with respect to intra-patient variation in baseline erythema, we recorded control erythema data outside the field (≥3 cms for all patients). Unfortunately, it did not prove possible to use symmetrically matched contra-lateral sites for all field configurations (e.g. midline axial skeletal fields, head and neck parallel opposed pair fields). We had anticipated that a normalisation or subtractive correction method would eliminate some of the intrinsic and extrinsic factors impacting on intra-patient variability over time. This did not prove to be the case, as several different correction methods clearly produced less coherent individual reaction plots and often meaningless pooled dose response plots. This situation was not improved when control values confined to contra-lateral sites were considered. In multi-variate regression analyses, both the

initial erythema reading at the test site and the peak erythema reading were very strongly dependant on the adjacent control erythema reading and in the FHDR study, equally strongly dependant on any adjacent test site reading (bolused or unbolused). These observations suggest that, as yet undefined, intrinsic loco-regional factors (and/or to a greater or lesser extent, extrinsic or systemic factors), contribute greatly to local erythema readings over and above the perturbing influence. (in this case, x-rays) Although erythema readings in the cancer control study patients were relatively stable, in a small number, major shifts occurred for uncertain reasons. Whether these shifts would be mirrored in other sites in the body (and/or in irradiated fields) must await further study. If large local changes in erythema can occur without a corresponding systemic change, then this may suggest that the use of adjacent or distant control values may be of extremely limited usefulness. Our finding, that approximately one third of patients in the FHDR and FLDR study had clear rises in control erythema readings outside the irradiated field, suggests the possibility of humoral factors mediating an inflammatory response and altering capillary blood flow, both inside and outside the irradiated field. Nyman 1995 (Thesis), found that bilateral internal mammary node irradiation (as opposed to unilateral) was associated with an enhanced erythema response, possibly reflecting the same phenomenon. Jolles & Mitchell 1947, has suggested that such a reciprocal vicinity effect may occur in irradiated human skin. Mortimer et al. 1991, also reported on x-ray effects outside irradiated fields in pig skin. He demonstrated reduced dermal clearance of ^{99}Tc-colloid between two 4x4 cm^2 test fields, some 8 cms from either field. In this instance the authors felt that the likely explanation related to the anatomy of lymphatics in the pig, which drain horizontally across the flank towards the limb roots. Although no patients enrolled in this study had overt skin involvement with tumour, underlying nodal or bony disease may also have had local effects on blood flow, influencing basal in-field and out of field erythema values. This hypothesis received some support from the reflectance data, in that pre-treatment reflectance scores in-field, exceeded control scores in approximately 20% of patients (FHDR and FLDR data). This difference however, was not statistically significant.

No significant rises in erythema at distant measurement sites were seen in the cancer study control group, demonstrating that the reciprocal vicinity effect is likely to represent only a local phenomenon and therefore may not be considered as a true abscopal effect. In a small number of patients in the FHDR study, it was apparent that the reciprocal vicinity effect was identifiable adjacent to one part of the field but not another. Furthermore, it was clear from both the FLDR and FHDR studies that only a subset of patients clearly demonstrated this phenomenon with a further subset maintaining very stable, unperturbed control values and another subset in whom apparently random variation of the control values did not permit any clear conclusions regarding

interactive effects. Whether the reciprocal vicinity effect is a result of differing local physiology or whether there exists a genetically distinct subset of "reciprocal reactors" is unknown.

4.6 Time Course of Erythema Reactions

In general terms, the onset, peak and recovery of erythematous reactions described in this study match those previously published in the literature (ICRP 1990; Coutard 1932). The trend towards a more rapid development of erythema in the hypo-fractionated data is consistent with the greater dose intensity (Gy/week) delivered in those patients. The rate of development of erythema showed significant variability, however in both studies tended to cluster at somewhere between 0.02 to 0.2 units per day. The observation that the 0.8 Gy/hr group tended to display higher rates of development of erythema for any given peak, (ie the opposite to the 20 and 32 fraction groups) is difficult to explain. Normally hyperfractionation may be thought of as biologically equivalent to lowering the dose rate, however in this instance it is likely that the low dose intensity (Gy/week) of the 20 and 32 fraction groups, produced low rates of development of erythema, and given the longer total duration of treatment, physiological mechanisms produced higher peak values. This implies time-dependent kinetics of release (or effect) for the humoral mediators concerned. This hypothesis is supported by the time courses of "out-of-field" reactions, which mirror those of the in-field reactions. Alternatively, longer treatment courses may be associated with the release of secondary mediators or other pigments (such as melanin) which might augment the peak radiation response.

Reaction durations also showed considerable variation, with longer durations usually being associated with higher peak erythema index, higher doses and in particular higher fraction numbers (20 & 32 fractions, FHDR group). In both studies a sub-population of patients clearly demonstrated a dip in erythema values during the evolution of their reaction at approximately two to three weeks from the initiation of treatment. A proportion of patients showed quite uniform rises and fall without significant "noise" or dips in the reaction at any stage. A further percentage of patients demonstrated test sites with no clear pattern of rise or fall in their reactions. Despite this heterogeneity in reaction pattern, the consistent presence of this "dip" which often extended to below baseline values (i.e. erythema index value <1), might suggest the presence of a vaso-constrictive influence during the evolution of the erythema reaction, or a bi-phasic reaction course. Fowler et al. 1963 demonstrated a dual wave of erythema in pig skin experiments. In single dose and five fraction experiments, this dip commonly occurred at approximately one month from treatment. These experiments employed doses and damage levels which were biologically higher than the present

study an therefore probably represents a different phenomenon. Hopewell 1986 also reported on two distinct peaks of erythema seen in the first 16 week period after pig skin irradiation. Based on isotope clearance studies and histological sectioning, Hopewell believes that the first erythema wave was indicative of a secondary inflammatory process resulting from the death of epithelial cells. Fowler comments that proliferative cell survival is not likely to be the only mechanism of damage to skin and underlying tissues and that other processes such as modification of vascular supply, could be contributing to the total effect. Our data also demonstrate that erythema production both in and out of field is clearly a complex event and is highly unlikely to be linearly (possibly even monotonously) related to basal cell kill.

The demonstration of an "inverse volume effect" (ie increasing effect for smaller field sizes) in the FHDR data may also have an explanation via the same mechanism. That is, fields with bolus (which were necessarily smaller as they represented subdivisions of a larger "parent" field) may have "added to" the reaction of an adjacent field subdivision without bolus via the reciprocal vicinity effect.

5 QUANTITATIVE VERSUS QUALITATIVE EVALUATION OF ERYTHEMA

In a large proportion of our FLDR and FHDR patients, the reflectance unit was clearly more sensitive in detecting the onset of radiation erythema than the naked eye. In both the "artificial erythema study" and the patient data set, there was a strong correlation between reflectance values of erythema and semi-quantitative reaction grades. Dawson et al. 1980 performed a similar artificial erythema study by asking observers to rank five pink tiles of different concentration of red pigment. Using a by eye ranking, the average ranking correlation coefficient was 0.48 (Kendall's tau). In contrast to this, the reflectance unit produced a ranking correlation coefficient with an average value of 0.97. The artificial erythema experiment performed in this study demonstrated marked inter-observer variation in tile grade assignment as well as significant intra-observer variation from day to day. This phenomenon was reproduced to an even greater extent in the FLDR study where massive overlap of erythema values over assigned grades by eye indicated a poor overall discriminative utility of the semi-quantitative scale.

Despite these problems with concordance of semi-quantitative versus quantitative measures of erythema, there remained a reasonable degree of correlation between these two parameters in the FLDR data set. Russell and co-workers 1994, reported a correlation coefficient of 0.33 for redness scores and clinical score of erythema in their post-mastectomy population. The nature of the correlation between semi-quantitative and qualitative evaluation of erythema was significantly dependant on sex, site and melanin index. Both the correlation coefficient and the slopes of the erythema index versus reaction grade regression

line, were greater for females versus males and non sun-exposed versus sun-exposed treatment sites. To a large extent, these two factors are related, in that females generally tend to have less heavy previous sun-exposure. A similar inter-relationship between these variables and treatment site is also inevitable in this data set. Quite why the eye should perceive sun-exposed sites and females as relatively pinker than males and non sun-exposed sites over and above reflectance readings is difficult to explain. As this study involved temporal variability in erythema, we must presume that the human eye (unlike the reflectance unit) cannot reliably recall the pre-treatment erythema and thus must rely on an intuitive estimate of previous erythema, erythema in the skin surrounding the treated field or untreated skin on a contra-lateral matched body surface. Any number of objections and possible biases may render these intuitive estimates or matching processes invalid.

The second major difficulty with semi-quantitative grading scales, results from the situation where one observer is uncertain as to whether a subtle skin reaction may be present. For this study we adopted a policy of specifying the quantal grade above that level of uncertainty, however, this is obviously open to criticism. Daniels and Imbrie 1958, discussed these problems in considerable detail and concluded that the human eye is an excellent device for sensing a quantal event but is inadequate in delineating graded differences of erythema or most importantly, changes over time. Dutreix and colleagues (1973), successfully utilised this quantal discriminatory ability to produce a dose response curve for human skin with relatively small patient numbers.

6 THE EFFECT OF MELANIN

The doses employed in both the FLDR and FHDR studies were not significant in terms of producing visible (to the naked eye) pigmentation. Reflectance readings however did discern a small but statistically significant peak in melanin values of approximately 10-15% over baseline. This peak is considerably lower than values measured by Turesson and colleagues and appeared to exhibit no consistent effects on multi-variate linear regression analysis apart from being positively correlated with initial erythema in the FHDR patients. This presumably reflects the higher sun exposure of these sites. Future in-vivo reflectance research could usefully concentrate on the possible effect of melanin on the measurement of the haemoglobin value, as variation in melanin either between sites or over time might critically effect response data. (UV or X-ray) The melanin index did however, appear to influence the perception of erythema by eye, in that skin in those regions with higher melanin indices tended to be assigned higher reaction grades. This supports the hypothesis that semi-quantitative subjective evaluation of erythema takes into account many other factors over and above simple skin redness. For example, it is possible that subtle changes in skin

texture, skin oedema and distribution of early erythema (e.g. peri-follicular), may also influence grading of skin reactions.

7 INFLUENCE OF PHYSIOLOGICAL VARIABLES ON PEAK ERYTHEMA

7.1 Pitfalls of Multivariate Linear Regression Analysis

In a noisy data set such as found in this study, the limitations of this technique should be considered. If 20 co-variates are tested, then one will often show significance (at a conventional level of $p<0.05$) purely by chance. A more rigorous test of significance in this situation is to divide the selected "significant" p value by the number of co-variates. In this instance the melanin content ceases to be significant for initial and peak erythema and field size likewise, for peak erythema. The effect of sex is retained for initial erythema, but lost for peak values. Several of the variables involved in this analysis were discrete, (eg. site, hair colour, sun-exposure) and are best considered by reduction to binary variables.(ie yes/no for each separate grade) If all are reduced in this way however, then the number of significant results, arising purely by chance, is increased. Other problems with the data set included non-normal distribution of the dependent variable for the FLDR patients (this related to a small number of outliers) and frequent multi-collinearity of co-variates. Best-subset regression analysis represents a method in which it is possible to select the best number of strongest co-variates for the development of a model. Ultimately a judgement based on strong uni-variate relationships and plausibility was combined with the linear regression results.

7.2 Physiological Variables

Apart from the effects of sex and measurement site on baseline erythema, our study has clearly demonstrated a dependence of peak erythema index on these and other factors. Tucker et al. (1992) hypothesised that patient characteristics such as age and smoking habits influenced the physiological response to radiation exposure. Our study demonstrated that treatment site, sex and age all influence the value of the peak erythema index. When analysed separately, previous ultraviolet exposure to the treatment site was also strongly negatively correlated with peak erythema index. Although this factor was not significant on sub-set analysis, this may simply reflect the small numbers involved in the study or the fact that previous sun-exposure was modelled as a single binary response and treatment site modelled as four separate binary responses, thus artificially increasing the probability of a significant result for treatment site over previous sun-exposure. Other authors have noted differences in erythema response to ultraviolet light with

body site. (Westerhoff et al. 1986). Rhodes and Friedmann 1992 demonstrated a significant difference in MED for UVB radiation of buttock skin in comparison to the mid-back for 36 dermatology patients and normal volunteers. They could not explain this finding on the basis of any difference in baseline erythema readings and postulated that differences in visual perception, epidermal thickness, mast cell behaviour, melanin concentration and blood flow might explain their observations. It would be expected that less sun-exposed sites, such as the buttock should have a lower MED, paralleling the observations in our study, with x-irradiation. In several patients treated in the FLDR study, where treatment fields spanned a dè-colletage area, a clear difference in visual erythema response was noted, suggesting that previous ultraviolet exposure may be acting, in addition to the effect of treatment site, in determining peak erythema index. No quantification of the effect of previous ultraviolet exposure on radiation response has been published in the literature, although the ICRP noted, (1990) that it is common practice for some radiotherapy departments to **reduce** the dose by approximately 10% for heavily sun-exposed areas. This is at odds with our findings, in that sun-exposed skin exhibited, on average, a lower peak erythema index reading. In arriving at a dose reduction recommendation, clinicians may in fact be observing end-points other than erythema (e.g. marked early inflammatory response which surrounds pre-malignant solar damage with radiation). Nyman 1995, (Thesis) demonstrated that older age, post-menopausal status and higher blood pressure were associated with a greater erythema peak. This is in contrast to our finding, of a negative correlation with age. This may be due to the fact that only internal mammary fields were used in that study, without any significant previous sun-exposure. That is, age may be acting as a surrogate variable for sun-exposure in our study. In common with our observations, he also found that pre-treatment erythema values were negatively correlated with the peak score. Clearly further studies are required to better identify the effects of factors such as those identified above and to quantify their contributions to inter- and intra-patient heterogeneity of radiation response.

8 DOSE RESPONSE DATA

8.1 FLDR

Although several different individual patient reaction parameters proved capable of generating dose response data from the FLDR study, peak erythema index proved to be the most robust parameter with respect to goodness of logit or even linear fit. This is in keeping with the observations of Turesson and others. Russell et al. 1994, utilised the rate of development of radiation erythema, however, was unable to generate a meaningful dose response function from relatively low patient numbers (n=44) and a low

range of doses per fraction and total dose. Duration parameters, area under curve and rate of development of reaction all correlated strongly with the peak erythema index. Rate of development of erythema in the FLDR patients was only weakly, positively correlated with measured dose, although there was a suggestion that for any given rate of development of erythema at the lower end of the low dose rate spectrum, a lower peak erythema index was reached in comparison to the higher dose rates employed in the study. The association of lower peak erythema indices for any given rate of development of erythema for the lower dose rates, may however, simply reflect the known sparing effect as the dose rate is lowered. Dose for dose, it did not prove possible to separate out the separate dose response curves for each dose rate in the FLDR study due to widely overlapping confidence intervals. Given the LQ predictions for the existence of only a modest dose rate effect over the range 0.8 to 8 Gy/hr and the significant inter-patient heterogeneity in this data set it is perhaps not surprising that this study failed to demonstrate a dose rate effect. Experience gained in the companion set of observations to this study, (semi-quantitative mucosal observations) (Appendix 4) indicated that a clear and coherent separation of separate dose response plots at different dose rates could be achieved. On that basis and having identified several of the major extrinsic and intrinsic causes of inter-patient heterogeneity, the FLDR peak responses were corrected (on the basis of the derived coefficients from the regression analysis) for the possible effects of age, site and sex. This did not improve the discriminatory power of either separate dose rate quantal plots or indeed allow a better logit fit for the pooled data.

The dose response plots for the 0.8 Gy/hr and 8 Gy/hr groups were negatively and marginally positively sloped respectively. This is indicative of the amount of noise in this data set and clearly requires greater patient numbers and possibly restriction of treatment site and technique in order to improve dose response coherence. Because of this inability to separate out dose response plots for each of the dose rate groups data was pooled for comparative analysis with the FHDR group.

It is of interest to note that no reaction parameter related to the semi-quantitative skin observations produced a meaningful dose response relationship. This must cast doubt on the ability of clinicians to reliably detect dose differentials in the order of 10 - 15%, from observations on skin in a day to day clinical setting. This has recently been confirmed in both radiation underdose and overdose incidents in the UK. (Ash & Bates 1994; Tobias 1992) where dose differentials of this order of magnitude occurred undetected over several years.

8.2 FHDR; Inverse Fractionation Effects

Dose response plot for the FHDR group (figures 63) clearly demonstrates an unexpected shift of the 20 and 30 fraction data to the left of the curves for the 5, 10 and 12 fraction data. As a complementary display method, dose-binned quantal response plots were also constructed, as the logit fits to quantal data do not display the raw distribution of values (figure 64). The binned quantal dose response data for FHDR patients again reveal the unexpected finding of higher responses at low doses per fraction for the 20 and 30 fraction data set, but not for the 5,10 and 12 fraction groups.

As a check of the validity of the dose binning procedure, individual dose bins in each of the studies were surveyed to exclude excessive skewness of dose distribution. This was noted in two dose bins in the FHDR study, however adjustment of the selected median values did little to alter the final plots. In addition, the quantal plots were constructed using several different cut points for peak erythema index (1.5, 1.6, 1.8). Again these procedures did little to alter the relative relationships of the data in each of the final plots. Although the 95% confidence limits for the 5, 10, 12 and 20 fraction groups are widely overlapping there is a clear reversal of the traditional left to right order of less fractionated to fractionated data. This observation is further strengthened by the 30 fraction data point, which in contrast to the 5, 10, 12 and 20 fraction groups, represents a single body site with highly uniform skin dosimetry.

In endeavouring to explain these aberrant results, several checks were made on the data. Firstly, patients having only one (rather than two or three) measurement sites were assessed, however limited numbers permitted no definitive dose response conclusions to be drawn in the 5 and 12 fraction groups. For the 20 fraction group there was no difference, dose for dose. For the 10 fraction group there was a slight suggestion that the 'single' area curve was shifted to the right.(ie less effect dose for dose) Although the reciprocal vicinity effect might explain some of the aberrant fractionation observations seen in this study, figure 73 still indicates a substantial under-prediction by the LQ model for observations where the reciprocal vicinity effect could not be operating. (30 fraction group and single test areas) Secondly, the data was checked for any significant differences in distribution of sex, sun-exposure, site and age with respect to the different fractionation groups. Apart from the greater presence of head and neck sites in the 10 fraction group, no significant differences were found and exclusion of the head and neck patients from the 10 fraction group made no material difference to the dose response curve.

9 ANALYTIC TECHNIQUES

9.1 F_e Analysis

This study employed a modified F_e plot analytic technique which was originally proposed by Scalliet (Thesis). This report has further adapted Scalliet's technique by utilising the incomplete repair equation for fractionated low dose rate radiotherapy from Dale et al. 1988. Since the dose rate modifying factor K depends on both α/β and μ, then the modified F_e plot methodology should be capable of deriving an α/β ratio for the data set when a value of μ is selected which produces the best linear arrangement of data points. It is clear from the F_e plot relating to this study, (fig 67) that the data in no way approximates a straight line. If the 20 and 30 fraction data is considered alone, then a negative α/β is suggested and conversely if it is excluded from the data set, then there is a reasonable approximation to a positive α/β in the region of 4-8, implying a very short t½ value (<0.15 hrs). This short t½ is not consistent with results from other human or pig data (table 16). The poor fit of the FHDR data to the linear quadratic formula is further supported by the Tucker and de Boer plots, where again non-linearity is clearly evident.

9.2 Direct Analysis

ABest analysis of the whole data set (5, 10, 12, 20 and 30 fractions) produced negative α values and therefore negative α/β ratios. This was not unexpected, due to the inverse fractionation effects seen in the raw quantal dose response plots. Of interest, the calculated repair half-times in these data sets still remain relatively low. Figure 74 displays the predicted fits for the entire data set and graphically demonstrates this effect in the low dose per fraction and low dose rate range. In this instance, a negatively sloped dose per fraction response for the FHDR data in the low dose per fraction range is conceptually implausible. (ie. greater effect, dose for dose as fraction size reduces is unlikely)

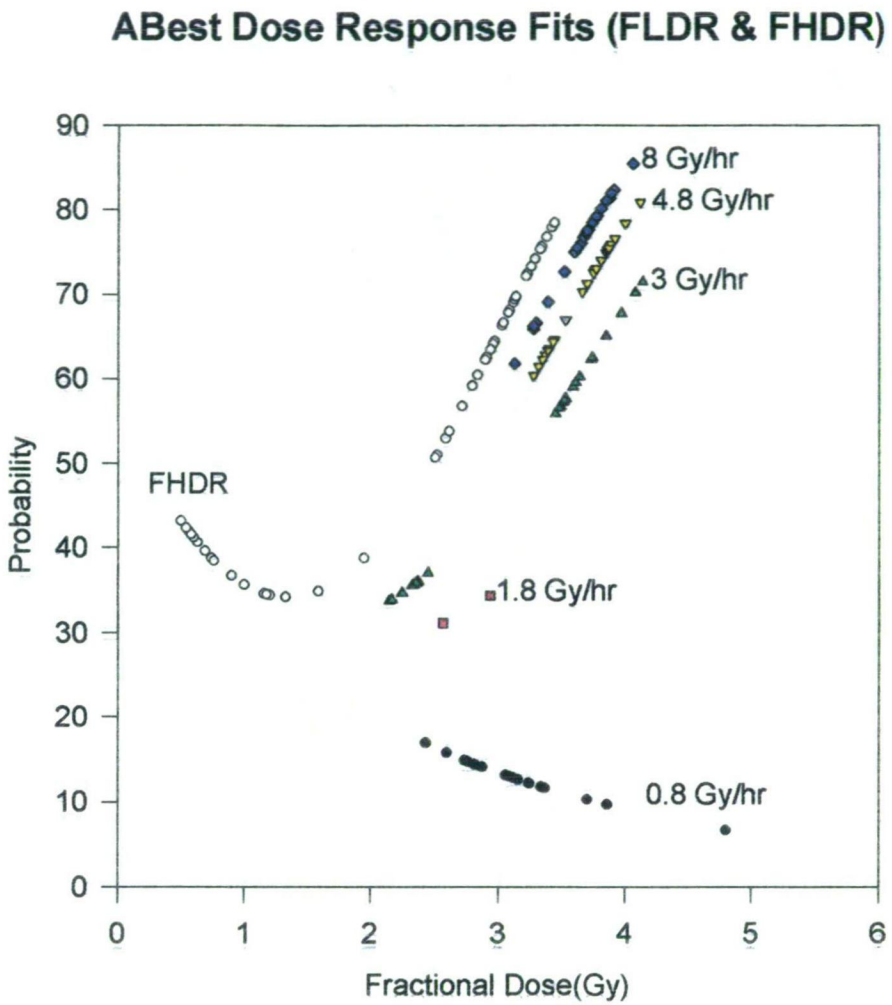


Fig 74 Abest predicted dose response fits for all FLDR and FHDR data.(excluding 32 fraction data)

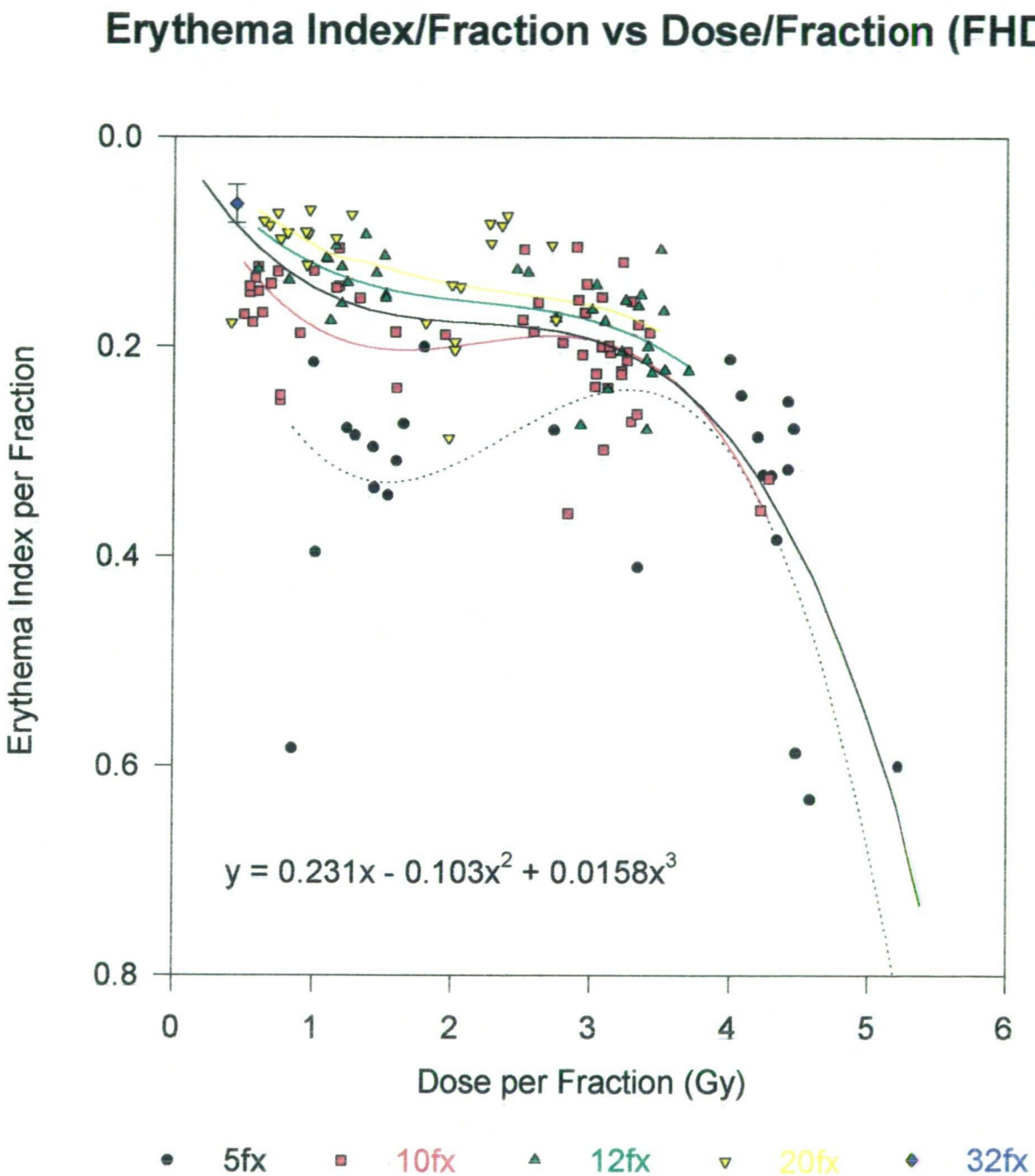


Fig75 Peak Erythema Index per Fraction vs Dose per fraction. Raw data for all the fractionation groups fitted with third order polynomials. Solid black line represents the fit for the whole FHDR data set, with equation given.

TABLE 16

Author	α/β ratio	Repair half time (hrs)	Comments
Turesson	18 - 34 7 - 11	0.3 - 0.4 1.1 - 1.3	long tmt duration short duration
Overgaard	10	-	
Bentzen	12	-	post- mastect
Wambersie	15	-	
van den Aardweg	3.5	0.27 ($\Delta t = 0.25$) 1.4 ($\Delta t = 4$)	pig 3 fractions
Chougule	9.1 - 9.3 7.5 - 7.9	- -	mild erythema intensive
Trott	4	-	Roentgen

Table 16 Reported α/β and t½ parameters

An additional problem in fitting our data to the LQ model is that the repair half time for acute erythema must be under 0.15 hours for an α/β ratio to be positive and in the range 4 - 8.

The LQ model assumes a mono-exponential repair model for the FLDR patients and complete repair between fractions for the FHDR patients. Turesson has found evidence of a bi-exponential repair process operating for acute effects in human skin. This may explain the very short repair half-times suggested by our data, in that it may only be the fast component of a multi-component repair process which is being measured. In addition, it is possible that the repair processes themselves are fundamentally influenced by dose rate. That is, repair mechanisms may be relatively saturated at high dose rate versus the low dose rate situation. Alternatively, repair processes may be different in the inter-fraction time period for the FLDR patients with respect to intra-fractionation repair processes. The modelling of these potential repair processes during and between low dose rate fractions must await further studies.

In summary, the inverse fractionation effects seen in the FHDR data set may have three possible explanations;

9.3 Study Design Flaw

An intrinsic study design flaw (ie. utilising individual patients, for two to three separate measurement points) could potentially enhance the effect of inter-patient heterogeneity, (by smaller effective patient numbers) and dilute the ability of the study to produce unique dose response curves. This is unlikely, as the composition of the 5,10,12, and 20 groups was similar for the important variables affecting radiation response. That same design flaw might potentially cause enhanced reactions at low doses per fraction via the reciprocal vicinity effect. This hypothesis is difficult to support, as the 30 fraction data clearly reinforce the trend seen in the other fractionation groups, and this group represent a totally homogeneous set of observations, with respect to site, sex, dose and sun exposure.

Another possible explanation for the inverse fractionation effect, is that we are measuring responses in the very early part of the sigmoid response curve and that noisy response data could be expected to produce an enhanced effect in one or two groups by chance alone. Again, this is difficult to reconcile with the quantal responses of the 5 and 10 fraction group, which are minimal in the low dose region. If the problem were just due to experimental error then one would expect this to be seen across all groups.

As all the low dose per fraction data was derived from un-bolused fields, it is possible that large dose gradients across the dermis and epidermis could explain the results. If one assumes that the target depth for radiation effect is 3-5 mm. rather than 0.07 mm. then the effect per fraction curve would fit a quadratic function quite nicely. We are unaware of any suggestion that the target site for radiation effects in the skin might be dermal or sub-dermal.

9.4 Physiological Theory

A further possibility is that there exist physiological process(es) which are more pronounced in a prolonged course of radiotherapy, (ie 20 and 30 fractions) resulting in greater erythema per total dose, than in shorter courses. Alternatively there may be a substantial physiological "threshold" which is exceeded only in the more prolonged courses. The finding that the prolonged courses were associated with higher peaks, for any given rate of development of erythema is also be consistent with this theory of time dependent erythema kinetics. However, this is not as plausible when the effects of time are accounted for, in the effect per fraction vs. dose per fraction plots. In this situation, the fraction number is equivalent to the total time of treatment. (This is not strictly the case, as treatment was given only 5 days per week, however this does not affect the results.) Figure 75 indicates that there is a gradation of inverse fractionation effects across the

groups. The 5 fraction group exhibits the greatest deviation from the LQ plot and the 20 fraction group, the least. (When this is translated to a dose response plot however, the multiplying effect of the fraction number "n" produces a much more marked left shift in the 20 fraction group over the 5 fraction group)

The application of the linear quadratic model to whole organ systems such as the skin requires the assumption that iso-effect always equates with iso-survival of the putative target cell. Nyman & Turesson 1994, has demonstrated (n=11) that accelerated fractionation is not associated with a greater or more rapid basal cell depletion than conventional treatment, despite a more rapid (but no more intense, erythema reaction). This is in contrast to clinically observed endpoints in mucosa where reactions are more rapid and severe with accelerated treatment. (personal comm. Prof J. Denham, re: Trans-Tasman Oncology Group Phase III Trial; Accelerated vs.Conventional Radiotherapy for Head and Neck Cancer) Whether this reflects a difference in cell cycle kinetics for skin vs. mucosa is unknown. Given that measured erythema is a complex end-point subject to a variety of physiological parameters, then the question must be asked as to whether iso-effect may indeed not necessarily relate to iso-survival. If for instance, the degree of measured erythema relates to the rate of basal cell kill and therefore the rate of release of any humoral mediators rather than the net cell kill, then this may render the application of the traditional LQ formalism invalid. Thus, studies such as these may in fact be measuring the "fractionation" sensitivities and "repair" capacities of physiological mechanisms rather than target cell kill per se. Further investigation of in-vivo cellular end-points, such as the hair cortical cell technique or basal cell density estimation (Nyman and Turesson 1994) may alleviate these problems.

9.5 Radiobiological Theory

Figure 75 displays the raw effect per fraction vs. dose per fraction data, with groups individually fitted by third order functions All groups show the "S" shape, however this effect is most marked in the shorter fractionation groups. Joiner et al (1993) have reported on radiation response to very small doses per fraction (FHDR) in mouse skin, lung and kidney via neutron top-up experiments. They showed that the LQ model significantly under-predicts for both acute and late effects, at fractional doses less than 0.6 Gy. Our data was also well fitted by the "Induced Repair Model" of Joiner et al. They suggest that this under-prediction may be due to the induction of repair mechanisms at higher doses per fraction. It is possible that our observed inverse fractionation effects (at low doses per fraction) may reflect the same phenomenon although this appears to start at doses of approximately 1.5 Gy

in our data which might argue against this conclusion. Joiner even suggests that a negatively-sloped dose per fraction response curve (figures 74 & 75) might be possible, if the magnitude of this effect were high enough. They also reported that this effect is more pronounced in shorter courses. (30 vs 20 fractions in mouse skin and 20 vs 40 fractions in mouse lung) Although our data also displayed this trend, this may simply have been due to the numerical effect of the fraction number "n", in its own right.

Alternatively, the inverse fractionation effect may represent greater cell kill per fraction, developing during the more prolonged courses. This implies a change in LQ coefficient(s) for longer total treatment times. In this dose per fraction range, this will be mainly due to a change in α. Turesson found no evidence for an increase in α with prolonged treatment time in her analysis. (Turesson and Thames 1989)

It is also possible that re-distribution of target cells into a more sensitive phase for the more prolonged treatments could explain the results. However the difficulty here, is that re-distribution would have to be confined to the low dose per fraction treatments only. This hypothesis is also difficult to support in viewing the time course of the 20 and 30 fraction data. One would expect a slower rate of development of erythema to be seen until recruitment occurred. (say at 2-4 weeks from start of treatment, Hopewell and van de Aardweg 1991; Turesson and Thames 1989) In fact, we see a constant rate of rise of erythema right from day 1.

9.6 **Relationship to Other Reflectance Data**

A comparison can be made with some of the sub-series published by Turesson where her data covers the same total number of fractions, fractions per week and fractions per day. This has included CREII (total number of fractions 10, dose/fraction 2.39 Gy), $CREVII_a$ (total number of fractions 20, dose/fraction 1.74 Gy), $CREVII_b$ (total number of fractions 20, dose/fraction 1.83 Gy), $CREVII_c$ (total number of fractions 20, dose/fraction 1.83 Gy) and 30 fraction data derived from a variety of series of longer duration. (Turesson and Thames, 1989) For this analysis, Turesson used an endpoint of a 50% increment in base-line erythema for the peak and our data is plotted in this fashion in figure 76. There is a marked disparity between dose response points for each of the data sets. Our FHDR data set displays shallower dose response plots. Whether this is a function of overall patient heterogeneity or differing responses of the reflectance units involved or both is impossible to say. Informal comparison of the response of the Gothenburg unit to ours, using "pink tiles" demonstrated that it displayed a greater "erythema" response than the Gothenburg probe.

No published reflectance data exists for fractionated low dose rate radiotherapy, however one of the series published by Turesson & Notter (Dose Rate III) may give some approximation of the dose rate effect as demonstrated by our data. In this experiment, weekly fractions of 7.2 Gy where given as 1 continuous fraction over 4 minutes or divided up into 3 subfractions given at 15 minute intervals over a total delivery time of 32 minutes. In crude terms this corresponds to a dose rate delivery of 14 Gy/hr. Turesson demonstrated a relative biological effectiveness for the prolonged treatment in relation to the short treatment time of approximately 1.1 for erythema. A similar RBE value maybe obtained by comparing our 10 fraction FHDR data with pooled FLDR data. Unfortunately, the dose per fraction used in our study did not exceed 5.5 Gy and therefore a direct comparison cannot be made, however at 4 Gy per fraction, an RBE of 1.25 has been observed for our data, which given our median dose rate of 4.8 Gy/hr for the FLDR group, is consistent with Turesson's observations.

10 FUTURE RESEARCH DIRECTIONS

Monitoring of radiation effects using complex endpoints such as erythema, in humans clearly requires a greater level of understanding of the mechanisms of erythema, humoral mediators of erythema and a better understanding of the extent of and reasons for inter- and intra-patient variability in radiation response. Little is known of the mechanisms of production of these various humoral factors in relation to ionising radiation. Young and Hopewell 1982 in isotope studies of irradiated pig skin, believed that the observed erythema was secondary to inflammatory processes relating to epithelial death. Physiological modulation of the expression of radiation damage may occur at a number of levels. Inflammatory mediators such as the ecosanoids and related compounds, TGF, TNF and other cytokines (Anderson et al. 1989; Black 1987; Imokawa and Tejima 1989; James et al. 1991; Kelfkens et al. 1990; Pentland and Jacobs 1991; Pentland et al. 1990) are likely to be involved in the expression of erythema. Hopewell et al, 1993, demonstrated that certain unsaturated essential fatty acids, which are known to be precursors of the ecosanoids, are able to modulate skin reactions to radiotherapy in the pig. Two essential fatty acids (gamma-linolenic and eicosapentanoic) where tested and found to produce amelioration of the erythema and/or moist desquamation component of the skin reaction, if administered during the time course of expression of the radiation damage. Other mediators such as histamine and epsilon amino caproic acid may also be involved in the production of erythema via altered vascular permeability (Eassa et al. 1973). From work performed in relation to ultraviolet induced erythema (Kelfkens et al. 1990) there is considerable evidence to indicate that a vaso-active mediator released from the epidermis, diffusing into and around dermal blood vessels, represents the most appropriate model for the generation of UV induced erythema. Further studies assessing the nature and kinetics of release

of these secondary mediators are required to better elucidate the link between cytology and physiology. Our observations regarding the higher peak erythema indices noted in females may relate to enhanced vascular reactivity secondary to hormonal effects. Larger clinical studies, directed at other acute and late endpoints (and tumours) are required to confirm a sex difference in radiation response. The negative correlation of peak erythema index with age may reflect reduced vascular reactivity associated with degenerative micro-vascular disease or possibly ageing effects due to cumulative ultraviolet exposure. Normal tissue radiation response in the elderly also requires further systematic study.

It is appropriate that future research aiming to model radiation effects in humans, utilises endpoints which are functionally and temporally closer to the presumed target cell in the skin. Techniques such as basal cell density counting (Nyman and Turesson 1994) and hair cortical cell counting (Potten et al. 1994) may provide better measures of in-vivo cell kill than the physiologically based endpoints used up until now. If endpoints such as these prove useful then it may be possible to identify radiation sensitive subsets in the population or alternatively more radio-resistant individuals and thus achieve some degree of dose or dose - volume individualisation of radiotherapy prescription and delivery. Attractive as these techniques may be, there are still several potential draw-backs in their utilisation. Unfortunately, direct visualisation in histological sections of basal cells do not clearly tell the observer what proportion of cells are abortively dividing or ultimately viable. Better histological or immuno-histochemical techniques are required to more clearly identify the subset of target cells which are dead or about to undergo either apoptopic or mitotic death. Hair cortical cell counting suffers from similar problems.

Possible future areas for further study in terms of radiation erythema include more formal assessment of the extent of the reciprocal vicinity effect, by mapping its anatomical extent around radiation fields. Demonstration of agents which may block some of the modulators of erythema (either topically or systemically) may also provide clues as to radiation erythema mechanisms and kinetics. This might include the topical administration of steroids, prostaglandin inhibitors or capsaicin. (Szallassi and Blumberg 1989)

A final research imperative arising from this data includes a more rigorous assessment of human skin response at low doses per fraction. An experimental design using uniform dose distributions over the dermis (eg. orthovoltage) would overcome objections related to build-up dosimetry. More patient numbers with better spread of dose per fraction data under 1 Gy in a consistent non-unexposed site would be important to confirm both our results and the in-vitro and animal work of Joiner et al. 1993.

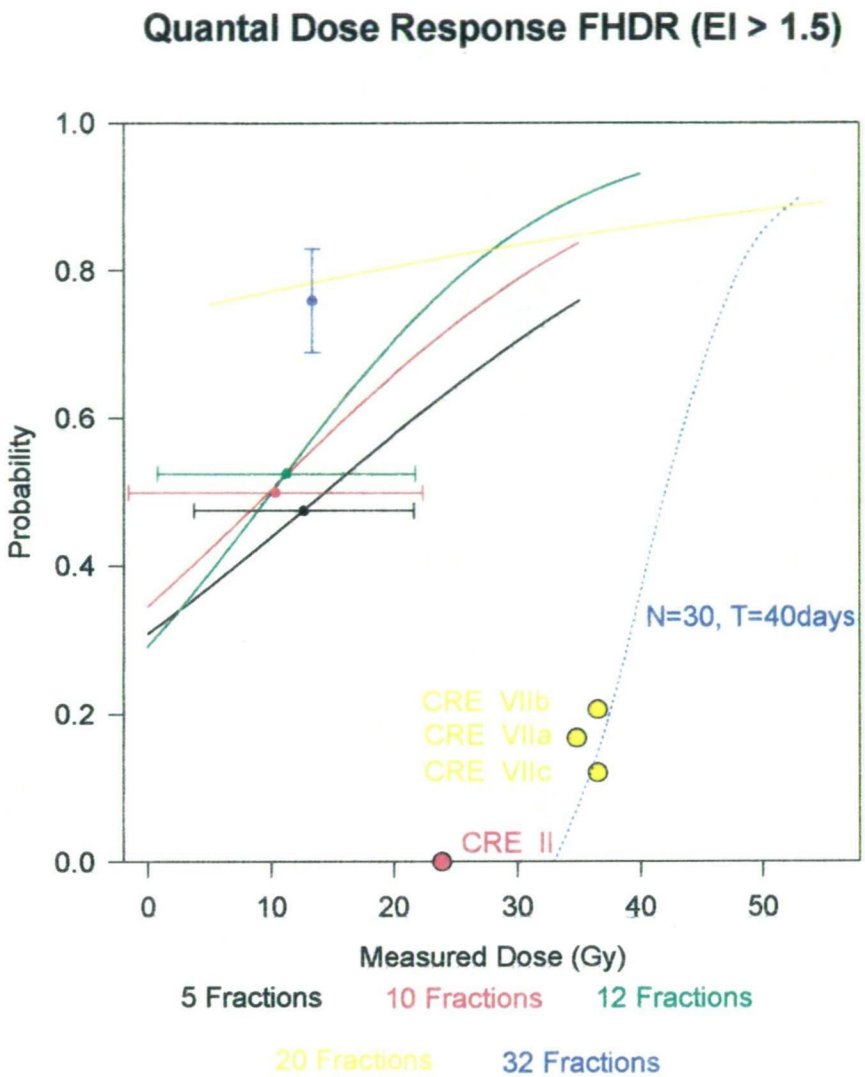


Fig 76 Logit fits for FHDR data using a quantal response cut-off of erythema index equal to 1.5 for peak erythema. Single dose response points for Turesson's data are displayed for comparison along with fitted dose response for her 32 fraction data. (figure 4, Turesson and Thames 1989)

11 CONCLUSIONS

■ Reflectance spectrophotometry is a sensitive and reproducible measure of human erythema, pre and post radiation exposure.

■ It exhibits considerably greater sensitivity and discriminatory power than semi-quantitative grading scales.

■ Human pre-irradiation and post-irradiation erythema shows marked inter-patient heterogeneity.

■ Initial erythema readings are influenced by sex, (males higher) and site. (sites in the upper half of the body higher)

■ Normalised peak erythema values give the best measure of radiation dose response.

■ Normalised peak erythema readings are positively related to; biologically effective dose, female sex and negatively related to age, melanin and previous sun-exposure to the site.

■ Erythema readings, adjacent to the field, display reciprocal vicinity effects, demonstrating the existence of local vaso-active humoral factor(s) responsible for both in-field and out-of-field effects.

■ Dips in erythema response may indicate the presence of vaso-constrictive component within 2 weeks of the start of treatment .

■ These findings strongly suggest that radiation-induced erythema in humans is not exclusively related to basal cell kill in the epidermis.

■ The FHDR data set demonstrate inverse fractionation effects at low doses/fraction, which may be due to different repair mechanisms operating at fraction sizes less than 1.5 Gy.

■ Both FHDR and FLDR data were **poorly** fitted by the linear quadratic model unless the prolonged treatment data and 0.8 Gy/hr data was excluded.

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APPENDIX

1

MERCY
we care

Newcastle Mater Misericordiae Hospital

Locked Bag 7
Hunter Region Mail Centre
N.S.W. 2310

HONE: (049) 211 211
AX: (049) 601 197

Dr C S Hamilton
Director
DEPARTMENT OF RADIATION ONCOLOGY
Telephone: (049) 21 1176

Ms Maree O'Brien
Research Co-ordinator
DEPARTMENT OF RADIATION ONCOLOGY
Telephone: (049) 21 1181

Ms Sue Wright
Research Nurse
DEPARTMENT OF RADIATION ONCOLOGY
Telephone (049) 21 1180

INFORMATION AND CONSENT FORM FOR PATIENTS PARTICIPATING IN THE HAIR COUNTING STUDY

This research project will use two methods to study the way in which radiation affects the skin. The first method will involve sampling hair which has been exposed to X-rays and examining it under a special microscope. It will then be possible to accurately measure the damage to the cells in that hair. This process has not been studied before and will have great importance in measuring the dose received to persons accidentally exposed to X-rays, (e.g. nuclear accident). We currently do not have any test which will measure this dose accurately in humans.

The second method used in this study will measure the amount of skin reddening appearing in the skin towards the end of and just after your course of radiation. This reddening (erythema) is a normal consequence of radiation treatment and relating this to the actual amount of cell damage occurring in the hair will enable better understanding of how humans respond to radiation, and ultimately better treatment methods.

Research procedures

The study requires that we take some hair samples just prior to your radiation treatment and on one further occasion during the course of your radiation treatment. On each occasion we will use a small electric epilator (hair removal instrument) to take the hairs from an area of approximately $2cm^2$ (about the size of a postage stamp). The sampling area will have an anaesthetic cream used before epilation and will be painless. The area of hair sampled will depend on where your radiation treatment is being given, and your doctor will explain details of this to you. The radiation therapy treatment given to you for your cancer has, as a normal side-effect, a greater or lesser amount of hair loss in the area being treated, and this is almost always only temporary.

The skin reddening effects will be studied using a simple machine called a reflectance meter. It uses harmless white light (not ultra-violet) to measure skin colour. A gentle small probe will rest on your skin for about 10 second for each reading. Each set of readings will take 5-10 minutes. You will feel absolutely nothing during the measurement. We will need to do one set of readings before treatment, twice weekly during the treatment sessions, and a further two to four readings after treatment has finished, necessitating either your return to the Department, or the visit of our Research Assistant to your home.

This study will have no effect on the radiotherapy treatment for your cancer, and your doctor will look after you in just the same manner as if you were not participating in this study. On one of your treatments we will perform a double-check of the X-ray dose received, using a tiny measuring probe placed on the skin. Again, with this instrument you will feel absolutely nothing, and it will have no effect at all on your treatment. You are completely free to withdraw from this measurement program at any time without prejudice to your medical care. Any skin reddening occurring during treatment will be a normal side-effect of the treatment and should be mild and short-lived. Your doctor or nursing sister will give you the appropriate advice and creams for this if required. The information collected on this study will include your name, address, medical record number, and all the measured parameters relevant to the scientific portion of the study. This will be stored, filed, and bound in our Data Manager's office, and only she and medical staff involved with the project will have access to this. Data stored on the computer will not have your name, address or medical record number. The data will be kept indefinitely for possible future comparison studies both at this Hospital and at others. At no point will your name or any personal details be released or published from this study.

Complaints

The Hospital requires that all participants are informed that if they have any complaint concerning the manner in which a research project is conducted, it may be given to the researcher or an independent person if preferred. (Quality Assurance Officer, HAREC, Room 315 Nurses Home, Royal Newcastle Hospital, Pacific Street, Newcastle, NSW 2300, Telephone (049) 266 432.

Consent

I agree to participate in the hair counting project, and give my consent freely. I understand that the project will be carried out as described in the information statement, a copy of which I have retained. I realise that whether or not I decide to participate, my decision will not affect my further medical treatment. I also realise that I may withdraw from the project at any time and not have to give any reasons for withdrawing. I have had all my questions answered to my satisfaction.

Signature Date

Information and Consent Form for patients participating in the Skin Reflectance Study

X-ray treatment is used to kill cancer and it is by this means that the treatment you are about to undergo, will improve your symptoms. We also know that some damage occurs in some normal cells. The skin (because it is the most visible body organ) is very useful to study this damage. This damage is seen as reddening or "erythema" in the skin, appearing towards the end of and just after a course of radiation. The treatment your Doctor has prescribed in your case will result in a very mild or even invisible reddening of your skin just over the area being treated.

We are able to study these invisible or mild skin effects using a simple machine called a reflectance meter. It uses harmless white light (not ultraviolet) to measure skin colour. A gentle, small probe will rest on your skin for about 10 seconds for each reading. Each set of readings will take about 5-10 minutes. You will feel absolutely nothing during the measurement. We will need to do one set of readings before treatment, twice weekly during the treatment sessions and a further 2-4 readings after treatment has finished. This will mean you will have to return to the Hospital twice per week in the 1-2 weeks following treatment.

This study will have no effect on your treatment and your Doctor will look after you in just the same manner as if you were not participating in this study.

On one of your treatments we will perform a simple double check of dose, using a small measuring probe placed on your skin. A small patch of material called "bolus" may also be placed over the part of the treatment field for each treatment to slightly increase the dose to the skin in that area. None of the technicalities will detract from the effectiveness of your treatment in any way.

You are completely free to withdraw from this measurement program at any time without prejudice to your medical care.

Any skin reddening occurring will be mild and short-lived. Your Doctor or Nursing Sister can give you hydrocortisone cream for this if required.

I ..... [signature] ..... have fully explained the nature of this Study to .....

I ..... [signature] ..... agree to participate in this research program, having read this information sheet and understand that I may withdraw at any stage without prejudicing any aspect of my medical care or follow-up. I understand that this study will be entirely confidential with respect to my personal details.

[Date] ..... / ..... / 199...

CONSENT TO TREATMENT FORM

I, ... the undersigned, hereby consent to my involvement in a research program into new therapies entitled "Low Dose Rate Teletherapy" conducted by the Department of Radiation Oncology, Mater Misericordiae Hospital.

I understand that my participation in this program will involve my treatment on the Caesium Teletherapy Unit at the Mater Hospital, involving treatments of several hours per day for up to two weeks.

The nature, purpose and contemplated effects of the program as far as it affects me have been fully explained to my satisfaction by my specialist and my consent is given voluntarily.

I am informed that no information regarding my medical history will be divulged and the results of any tests involving me will not be published so as to reveal my identity.

I understand that my decision to become involved in the program will not affect my relationship with my medical advisers in their management of my health. I also understand that I am free to withdraw from the program at any stage.

I understand that this program has the approval of the Regional Research and Ethics committee.

. . . .

(Signed) (Witness) (Date)

I, ... have explained the nature, purpose and contemplated affects of this program to . . . . and have also explained the randomised process. I am satisfied that he/she understands what he/she has been told and that he/she participates of his/her own violation.

. . . .

(Signed) (Date)

APPENDIX

2

Australas Radiol 1990; 34: 241-246

Low Dose Rate Teletherapy Using a Telecaesium 137 Unit Radiobiological, Physical and Clinical Considerations

S.G. COOPER, A.P. CARDEW, S. FERGUSON, D.J. JOSEPH, C.S. HAMILTON AND J.W. DENHAM
Department of Radiation Oncology
Newcastle Mater Misericordiae Hospital.
A.R. WILLIAMS
Australian Nuclear Scientific Technology Organisation
Lucas Heights.

ABSTRACT

Low dose rate teletherapy aims to combine the supposedly superior results obtained with low dose rate implants with the convenience and staff protection characteristics of external beam therapy. Previous investigators have used telecobalt units to produce dose rates of 1.1 to 1.8 Gy/hr to treat in daily sessions lasting 6-10 hours to total doses of 60-70 Gy. These studies have not discounted the possibility that much of the advantage of interstitial implants results from the low dose rates used per se, and from the fact that the total dose is delivered in a short overall time. The relationship between total dose, dose rate and volume giving normal tissue and anti-tumour effects, however, remains ill-defined. At the Newcastle Mater Misericordiae Hospital a Caesium teletherapy unit has been modified to treat at low dose rates and a study has been designed with a view to establish which permutations of total dose and dose rate are isoeffective for acute mucosal and acute skin reactions in the dose rate range between 0.8 and 9.6 Gy/hr (1.3 and 16 cGy/min).

THE DOSE RATE EFFECT – BASIS FOR ENVISAGING A THERAPEUTIC GAIN

Dose rate is one of the key factors in determining the biological effect of any given total dose. In general terms reduction of dose rate and consequent elongation of exposure time reduces the biological effect of a given dose. Interestingly, in spite of this reduction in effect, it has been suggested that the therapeutic efficacy of intracavitary and interstitial brachytherapy is as much due to an improvement in therapeutic ratio caused by treating at low dose rates as to the dose differential between tumour and normal tissue that is commonly achieved by these techniques. It has even been suggested that some of the impressive results produced by the first teletherapy units in the 1920's and 1930's were due more to the prolonged exposure times required for treatment than the physical characteristics of the beams achieved!

This proposal has some foundation in experimental fact. According to Hall (Hall 1988), reductions in effectiveness due to progressive reduction in dose rate occur largely because repair (predominantly sublethal repair) takes place during exposure itself. As dose rate decreases the amount of repair that is possible during exposure increases until all repairable cellular lesions do repair. Once this happens the usual "shoulder" to the cell survival curve disappears altogether and cell killing is effectively exponential. While this effect does not in itself lead to a therapeutic gain, the factors that influence repair of sublethal cellular damage certainly may. Cellular hypoxia is one of these (Hall and Cavanagh 1969). Chronically hypoxic cells, which exist in abundance in many human tumours, appear to be less efficient in repairing sublethal injury (Nias 1988). As a result, the differential effects of radiation on well oxygenated and hypoxic cells reduce as dose rate decreases, leading to a reduction in oxygen enhancement ratio and a potential therapeutic gain (Fu and Phillips 1975) (Hill and Bush 1973).

Another important factor to influence sublethal repair is the intrinsic repair capacity of the target cell itself. The cells of late reacting normal tissues characteristically shed sublethal injury more effectively than the cells that govern the response of early reacting tissues. Reductions in the rate at which radiation is applied will therefore be expected to result in differential sparing of late reacting tissues because these are able to repair sublethal injury more efficiently. The situation is summarised by Steele (Steele *et al* 1989) in graphical form (Figure 1).

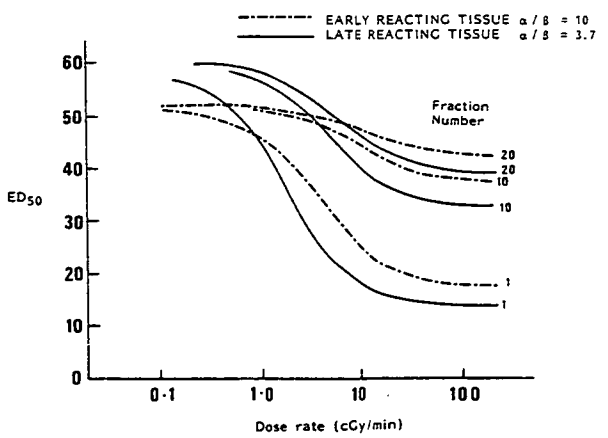

FIGURE 1 – The dependence of biological effect (in this case ED50) upon dose rate for early and late reacting tissues according to whether treatment is given continuously or divided into 10 or 20 equal fractions (modified from Steele *et al* 1989).

According to Hall (Hall 1988), the application of radiation at low dose rate is analogous to the application of an infinite number of very small fractions at high dose rate. The reasons for suspecting that low dose rate irradiation may be associated with a therapeutic advantage are there-

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fore the same as those for suggesting that **hyperfractionation** may be beneficial.

These are not the only biological reasons why brachytherapy has been mooted to be more effective than conventional fractionated irradiation in human tumours with rapid cell turnover times. Repopulation of tumour clones during a fractionated course of treatment is now being cited as a potential cause for treatment failure. According to Withers *et al* (Withers *et al* 1988) there is some evidence that this process may be responsible for escalating dose requirements when the treatment course is protracted over periods of more than four weeks. One reason that brachytherapy may be successful, therefore, is because treatment is rarely protracted over more than two or three weeks.

Another factor of potential importance is an effect that has become known as the "inverse dose rate effect" (Hall 1988). When the duration of exposure exceeds 1-2 cell cycle times of the tissue under consideration, cell cycle progression sensitivity phenomena begin to dominate the response. Accumulation of cells due to progression block at G2 would be expected and would result in a relative sensitisation of these cells. Cell killing of rapidly cycling populations would therefore be expected to increase at low dose rate with possible differential sparing of tissue composed of slowly proliferating cells.

Unfortunately, despite the encouragement that has come from the laboratory that treatment at low dose rate itself might be responsible for a therapeutic gain in the clinic, the matter remains conjectural. The concept that hyperfractionation of dose is associated with a therapeutic gain in head and neck cancer has received some support from controlled clinical trials (Horiot *et al* 1989). The notion that acceleration of the treatment may be beneficial has also received some support from uncontrolled studies (Gray 1986). The gain that might be expected from treatment at low dose rate, however, has not been clearly shown in any study.

Teletherapy using dose rates in the (low dose rate) brachytherapy range was developed at the Henri Mondor University Hospital, Creteil, France, by Bernard Pierquin in the hope of extending the perceived advantages of brachytherapy to the many clinical situations where brachytherapy is impossible. After preliminary pilot studies with advanced head and neck cancer, Pierquin's group embarked on a trial comparing low dose rate external beam therapy using a modified Cobalt teletherapy unit with conventionally fractionated external beam therapy (at high dose rates) for moderately advanced oropharyngeal tumours. This study was first reported in 1985 (Pierquin *et al* 1985) and subsequently updated in 1987 (Pierquin *et al* 1987). Highly significant differences in local recurrence rate were observed between patients treated by low dose rate irradiation (5/32, 16%) and patients treated with conventional high dose rate fractionation (20/33, 61%). 44% of patients in the low dose rate arm versus 24% in the conventional treatment arm were alive with no evidence of disease at the time of the latter report.

Unfortunately, however, difficulties in interpretation arise because patients were not truly randomised. As a result the two treatment arms were not entirely comparable with respect to some potentially important variables. For example, there was a higher proportion of node-positive patients in the conventionally fractionated arm, while in the low dose rate arm the primary site was boosted with an Iridium-192 implant in a higher proportion of cases permitting higher overall total tumour doses to be achieved. Biological considerations such as the effect of the split in the low dose rate treatment course, and the range of dose rates used, have added further complexity to the interpretation of the results.

Perhaps of even more importance is the fact that no clear indication of a **therapeutic gain** emerged from this trial. While the tumour responses achieved were indeed impressive (and possibly quite unexpected) these results were accompanied by five major necroses and two treatment related deaths. This, of course, raises the question that a higher biologically effective dose had been used in the low dose rate treatment arm of the trial. Indeed, despite the extensive experience gained with brachytherapy this century, and the more limited experience gained with low dose rate teletherapy at other centres (Kuipers 1978, Wilson 1978), it remains difficult to predict what modification of total dose is necessary to avoid exceeding the tolerance of **any** normal tissue if dose rate is altered either by circumstance or design. In the absence of this fundamental information on normal tissue effects, it is therefore hardly surprising that the question of therapeutic gain from treatment at low dose rate has not been resolved.

The reasons why there is this dearth of information are not hard to understand. The maximum doses that can be delivered without unacceptable risks of late damage for interstitial/intracavitary therapy are a complex function of total dose, volume and dose rate. These are in addition to factors such as source type, configuration and anatomical site. In attempting to resolve these difficulties, Paterson, from Manchester, produced a guide indicating that reductions in total dose should occur if an implant geometry unintentionally were to deliver a reference dose above 0.35 Gy/hr (Paterson 1963). The Paris group suggested more recently, on the other hand, that no such dose reduction is required in the range 0.3-1 Gy/hr (Pierquin *et al* 1973). While debate continues over dose rate modification requirements for interstitial therapy, some progress has been made for intracavitary therapy. Hunter and Stout, from Manchester, have produced data from a well-designed clinical trial indicating that there is a necessity to reduce overall dose when after loaded gynaecological sources are used to treat cervical cancer at 1.4-1.8 Gy/hr (2.3 – 3 cGy/min) when compared to the standard use of manually placed radium sources at 0.53 Gy/hr (0.9 cGy/min) (Hunter and Stout 1989, Stout and Hunter 1989). The dose modifying factor is in the range between 10% and 19% which is significantly lower than predicted from models based on the linear quadratic equation. A dose rate modifying factor for the development of pneumonitis has also been suggested by data from bone marrow transplantation programs using preliminary total body irradiation (Barrett and Depledge 1982). Quantification of this effect, however, has been confounded by the high incidence of pneumonitic processes due to other causes such as graft versus host disease and opportunistic infection.

In summary, therefore, while some encouragement has come from the laboratory, the issue of a therapeutic gain in the clinic from the use of low dose rates remains unresolved. Perhaps even more important, however, is the fact that there remains a dearth of basic clinical data concerning the effects of dose rate on normal tissue tolerance. Until this information becomes available the question of therapeutic gain can never be satisfactorily answered.

It was these uncertainties and the availability of an aged but functional Siemens Caesium teletherapy unit that

lead to a project at the Newcastle Mater Misericordiae Hospital being set up to examine the dose rate effect between the low and high dose rate ranges for acute reactions in skin and oropharyngeal mucosa in human beings.

ESTABLISHMENT OF A LOW DOSE RATE TELETHERAPY FACILITY AT THE NEWCASTLE MATER MISERICORDIAE HOSPITAL

The Caesium Teletherapy unit at the Newcastle Mater Misericordiae Hospital normally operates at treatment distances of between 20-30 cm SSD and delivers incident dose rates of greater than 1 Gy/min. Because the 50% depth isodose line is less than 5.5 cm deep at these SSD's the use of the machine is confined to the palliative treatment of relatively superficial primary and secondary tumours. Modification of the unit for low dose rate use has involved the construction of a set of collimation cones from low melting point alloy (as illustrated in Figures 2 and 3) to shape the beam at extended SSD and minimise the penumbra of the resulting beam. A series of beam flattening attenuating filters are used to achieve flat beams at 80 cm SSD and produce instantaneous dose rates between 0.5 and 5.0 Gy/hr at 7 cm depth in tissue (approximately 65% isodose, depending on field size). These criteria have been laid down to meet the requirement that heterogeneity of total absorbed dose in the irradiated volume not exceed ± 5% using parallel opposing fields up to a separation of 14 cm. The profile of the beams produced by these means is shown in Figure 4 and its dose characteristics are shown in Figure 5.

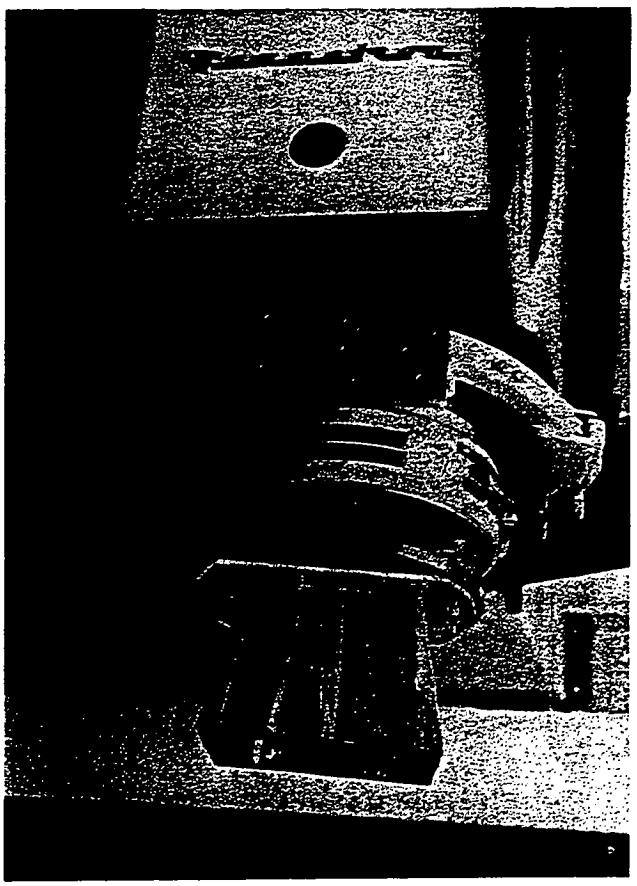

FIGURE 2 – Modifications to Caesium teletherapy unit for treatment at low dose rate. A diverging low melting point alloy collimation cone is fastened to a perspex mount which has a "pocket" to house a lead attenuating filter.

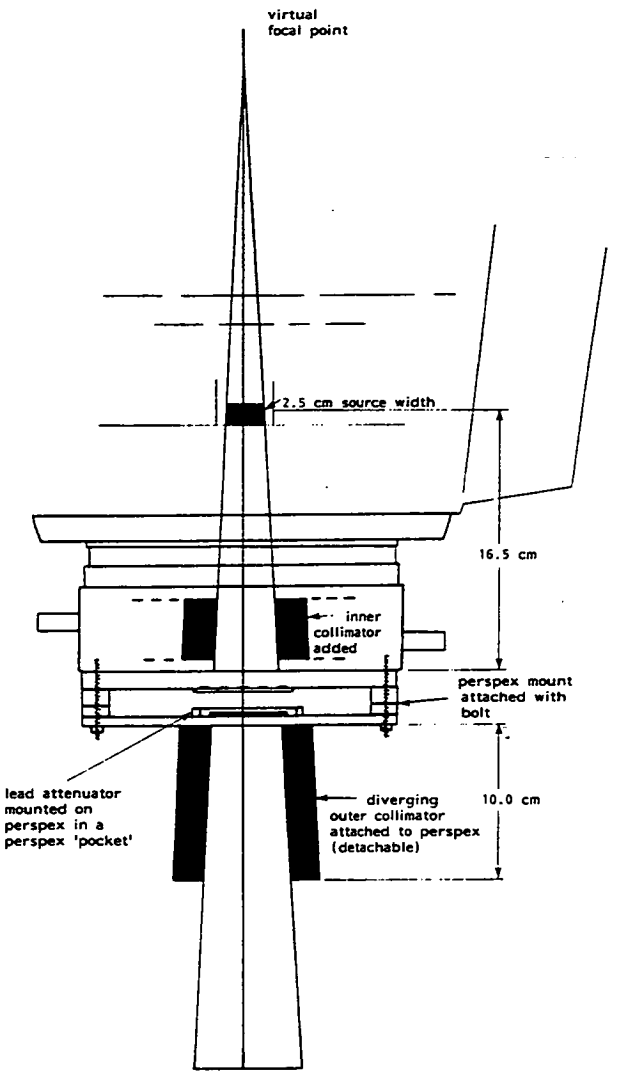

FIGURE 3 – The Caesium teletherapy unit "head" illustrating the design and positioning of the "inner" and "outer" diverging low melting point alloy collimators in relation to the caesium source.

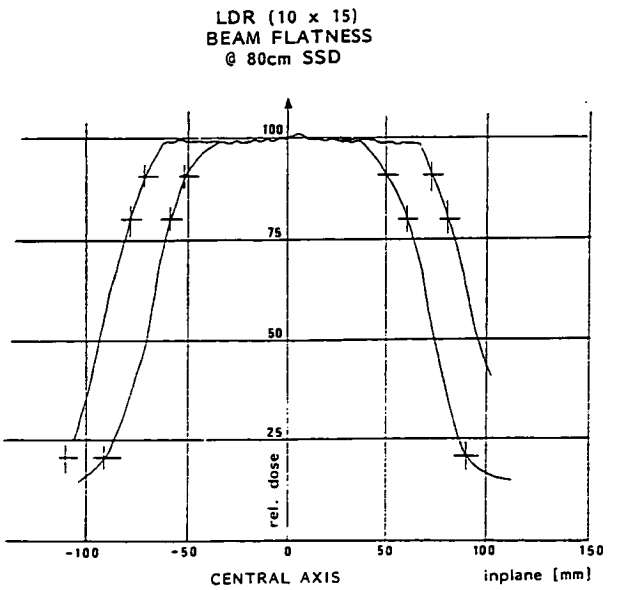

FIGURE 4 – Beam profile for a 10 x 15 cm cone showing the flatness of the beam and its penumbra at 80 cms SSD.

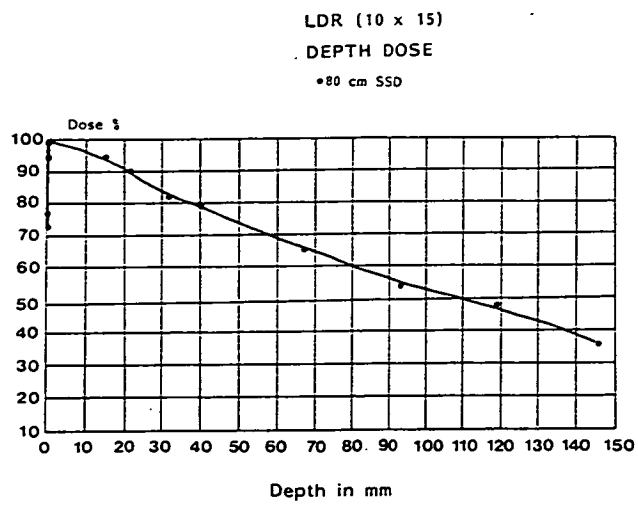

FIGURE 5 – Depth dose characteristics of a 10 x 15 cm field at 80 cms SSD. Peak dose is estimated to be 0.5 – 0.7 mm below the surface.

Because treatment times between 1 and 5 hours per day are projected in the dose rate study proper, careful consideration has been given to patient comfort and immobilisation during treatment. The best conditions for treatment have been achieved with patients reclining on a modified dental couch with hourly rest breaks allowed (Figure 6). Immobilisation in head and neck cases has been achieved by using customised polystyrene head rests with suitable Velcro strapping. The earlier patients were monitored remotely by closed-circuit TV and their position checked against a standard. Currently, a series of optical proximity switches are being trialled so that arrays in two planes can confirm patient position and switch off the unit as required if the patient strays from the prescribed field setting for more than a few seconds. This will permit the measurement of elapsed time of the patient outside of the optimal treatment position. A TV equipped with a VCR and a radio-cassette are provided for entertainment during treatment.

FIGURE 6 – A patient in treatment position. A modified dental chair with a customised headrest is positioned on a lifting palette. A television with video is available for entertainment and contact is maintained via a microphone and closed circuit television.

Beam direction is established by front and back pointers and from the light beam produced by the unit. The treatment itself is supervised on closed circuit TV.

In a feasibility study to establish techniques five patients with incurable head and neck cancers have been treated. Three patients with squamous head and neck cancer have been treated using opposing lateral fields. Two of these were treated to midline doses of 30 Gy in ten daily treatments lasting approximately 5 hours per day at 0.6 Gy/hr (1 cGy/min) using lateral fields opposed at hourly intervals and one has received a total midline dose of 45 Gy at 0.6 Gy/hr. Two of these patients have had lasting partial response to treatment while one showed little response.

Two patients have been treated with direct fields. One of these patients had recurrent basal cell carcinoma encircling the trachea and invading the superior mediastinum after several attempted resections and received a dose of 30 Gy at D4 cms in 10 daily sessions of 5 hours per day at 0.6 Gy/hr. Skin healing has taken place and his tumour remains controlled eight months after treatment. The other patient had recurrent melanomatous nodal disease in the neck following resection and received an incident dose of 30 Gy in 10 daily sittings at 0.6 Gy/hr responded entirely but relapsed two months later. Because evidence of disease at other sites had not appeared at that stage he was offered retreatment using conventional external irradiation using 6 MV X-rays at 2 Gy/day. He received an additional total incident dose of 50 Gy before skin reactions drew treatment to a close.

It has been noted that skin and mucosal reactions have been characterised by a specific time course: commencing during the first week of treatment, building to a peak at 2-3 weeks after the commencement of treatment, and resolving rapidly and entirely by 6 weeks. Severity of reactions have not exceeded Grade III in the mucosa and Grade II in the skin according to RTOG/EORTC criterial (Table 1) in any of the patients.

TABLE 1

EORTC/RTOG Toxicity Criteria

SKIN	0	No change over base line
	* 1A	Follicular, faint erythema/epilation/dry desquamation/decreased sweating
	* 1B	As above but confluent bright pink erythema
	2	Tender or dusky erythema, patchy moist desquamation/moderate oedema
	3	Confluent, moist desquamation other than skin folds, pitting oedema
	4	Ulceration, haemorrhage, necrosis
MUCOUS MEMBRANE	0	No change over base line
	1	Injection; mild pain not requiring analgesics
	2	Patchy mucositis which may produce an inflammatory serosanguinous discharge/may experience moderate pain requiring analgesics
	3	Confluent fibrinous mucositis/may include severe pain requiring narcotics
	4	Ulceration, haemorrhage or necrosis

* This distinction is a local one and therefore represents a modification.

In addition to perfecting the techniques for the dose rate study outlined below, the feasibility study has shown the potential of very modest doses of radiation given in this way to produce tumour responses that appear to be just as satisfactory as those produced by intensive chemotherapy or conventional fractionated palliative therapy.

STUDY DESIGN ADOPTED TO EXAMINE THE DOSE RATE EFFECT ON EARLY REACTING NORMAL TISSUES

The present study has been designed to address the effect of dose rates between the conventional brachytherapy range and the high dose rate external beam range on normal acutely reacting tissues: the skin and oropharyn-

geal mucosa. Late normal tissue effects have not been addressed in the study design and nor has anti-tumour efficacy.

As shown in Figure 7 isoeffect curves, calculated using a modification of Dale's formalism (Dale 1985), change significantly in the low and 'medium' dose rate ranges for both acute and late reacting tissues. The major question to be addressed in the present study is how reliable these estimates actually are. Six different points on the predicted isoeffect curves have been selected (according to Figure 7) to test the veracity of these predictions or serve to provide data for fresh predictions.

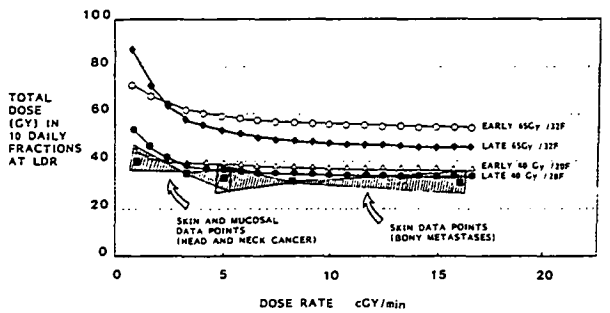

FIGURE 7 – Curves for early and late reacting tissues for effects predicted to be equivalent to 65 Gy in 32 fractions over 6.5 weeks and 40 Gy in 20 fractions over 4 weeks using conventional (high dose rate) teletherapy according to Dale's formalism (Dale R.G. 1985). The data points addressed in the present study are enclosed in the shaded area. The accuracy of Dale's formalism will be predicted by the isoeffectiveness of the square data points.

Consenting patients with extensive and incurable head and neck cancer (according to the criteria laid down in Table 2) and metastatic bone cancer are allocated according to a central composite group design. According to this design patients are treated for head and neck cancer to 38 Gy at 1.8 Gy/hr in 10 daily sessions until sufficient normal tissue data are accumulated to characterise the time course and severity of reactions at this particular permutation of dose and dose rate. Subsequent patients are then allocated to receive doses 10% greater or less than the total doses predicted to be isoeffective at 0.8 Gy/hr and 3.0 Gy/hr (i.e. 40 Gy and 36 Gy) in 10 daily sessions. Skin and mucosal reactions are scored on a weekly basis by two observers independently according to the EORTC/RTOG criteria (see Table 1). Tumour response is documented by weekly calliper checks and, where indicated, by CT and or EUA.

TABLE 2

Patient Selection Criteria

Head and Neck Cancer

(1) Patients with squamous carcinoma of the head and neck that fulfil the RTOG modification of the AJC stage grouping criteria in the categories IVB, C and D and V *, excepting oral cavity tumours and laryngeal tumours that may be amenable to surgery.

(2) Patients under 80 years of age with an anticipated survival of more than 8 weeks.

(3) Patients who do not have intercurrent illnesses such as dementia, severe epilepsy, incontinence, severe musculo-skeletal disorders, that are likely to make treatment lasting several hours each day difficult.

(4) Patients who do not have severe dental caries requiring major dental work prior to treatment.

(5) No prior treatment (radiation or chemotherapy).

(6) Written informed consent to treatment on protocol.

Bony and Soft tissue metastases

(1) Proven symptomatic metastatic cancer involving sites that can be encompassed satisfactorily by our modified caesium beam at 60 or 80 cms SSD.

(2) A disease site that has not been previously irradiated, is not situated in a pressure area, and in which there is no evidence of skin or subcutaneous involvement by tumour.

(3) The patient has not received Adriamycin, Actinomycin D, or Bleomycin containing regimes, or is being concurrently treated with cytotoxic chemotherapy or other agents that may modify radiation response.

(4) Ability to lie still for a period of up to 1 hour.

(5) Written informed consent for treatment on protocol.

Table 2: Criteria for study inclusions

* Because it is anticipated that some patients with RTOG IVB, C, D and V disease will respond favourably to this protocol, it is planned that further therapy using "conventional" teletherapy techniques or implantation will be offered to such patients after normal tissue reactions have subsided – as in the Creteil study.

In a separate arm of this study a similar set of observations are conducted for skin reactions in patients with bony metastases. In this instance, however, the starting dose is 32 Gy at 4.8 Gy/hr (8 cGy/min) and isoeffective conditions are to be looked for at 1.8 Gy/hr (3 cGy/min) and 9.6 Gy/hr (16 cGy/min).

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RADIOTHERAPY & ONCOLOGY

Acute reaction parameters for human oropharyngeal mucosa

J.W. Denham*[a], C.S. Hamilton[a], S.A. Simpson[a], M.Y. O'Brien[a], P.M. Ostwald[a], T. Kron[a], K.B.G. Dear[b]

[a]*Radiation Oncology Department, Newcastle Mater Hospital, Waratah, NSW 2298, Australia*
[b]*Statistics Department, University of Newcastle, Callaghan, NSW 2308, Australia*

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Abstract

The purpose of this study was to determine the influence of changes in dose rate over the range 0.8–240 Gy/h on acute oropharyngeal mucosal reactions in human subjects, and to estimate the values of the important parameters that influence these reactions. Sixty-one patients requiring radiotherapy to palliate incurable head and neck cancer were treated on a telecaesium unit, using opposing lateral portals to total midline doses, varying between 30 and 42 Gy in 10 daily fractions over 2 weeks, at dose rates of 0.8, 1.8, 3.0 and 240 Gy/h according to a central composite study design. The severity and time course of reactions were charted at least twice weekly for each patient, using the EORTC/RTOG acute mucosal reaction grading system. Duration of reaction at each grade was observed to provide a more sensitive reflection of effect than the proportion of patients reaching any particular reaction grade. Analysis of duration by direct and indirect methods suggest α/β ratios in the range 7–10 Gy and half-time ($t_{1/2}$) values in the range 0.27–0.5 h, if mono-exponential repair kinetics are assumed. The $t_{1/2}$ values are short and raise the question as to whether the repair kinetics of this tissue are well described by a mono-exponential function. Further prospective studies involving multiple daily fraction treatment regimes delivered at high dose rate, in which interfraction interval is deliberately varied, are needed to find out whether the parameters derived from this project are applicable to fractionated treatment courses at high dose rate.

Keywords: Mucosa; Low dose rate; High dose rate; Alpha/beta ratio; Repair kinetics

1. Introduction

Over recent years, considerable progress has taken place towards the development of models that will be helpful to clinicians in estimating the effects that are likely to be observed for many commonly used permutations of total dose, fractionation, and dose rate. The incomplete repair model, for example, provides very reasonable fits to many sets of laboratory and clinical data [23,24]. Of course, few clinical data are generated with the specific intention of defining the values of the parameters of the predictive models, therefore much of what is known is based on the re-analysis of historical clinical data [23]. In the past few years, increasing interest in the use of accelerated fractionation schedules delivering more than one treatment fraction per day has drawn attention to the importance of the rate at which repair occurs between fractions [10,14,15,18,28]. The process, which for operational purposes is considered, mono-exponential in the incomplete repair model [22], is best described by its half-time ($t_{1/2}$). Unfortunately, a dearth of relevant clinical data has prevented suitable values for this parameter from being derived with any certainty for any acute or late reacting human tissue. At the present time, therefore, a better understanding exists concerning appropriate values for α/β than for $t_{1/2}$ [23]. Increasing use of accelerated and hyperfractionated treatment regimes in locally advanced head and neck cancer has also drawn attention to the importance of the severity of acute mucosal reactions in determining treatment tolerance [18,25].

The fractionated low dose rate (FLDR) teletherapy project at the Mater Hospital, Newcastle, Australia [5],

* Corresponding author, Tel.: +61 (0)49 211177; Fax.: +61 (0)49 602566.

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was therefore designed in response to the need for more prospective clinical data to describe α/β ratio, and $t_{1/2}$ parameters, for acute mucosal reactions as well as the dose rate effect itself. This has been done by examining reactions caused by identically fractionated treatment regimes, delivered at low to medium dose rates (i.e., under 10 Gy/h), and at high dose rate (240 Gy/h). This report describes the indirect and direct quantal analysis of these data and discusses the implications and remaining uncertainties of our results.

2. Patients, materials and methods

2.1. Patients

The 61 patients enrolled for treatment on this project had locally advanced squamous cancer of the head and neck (RTOG modification of AJCC criteria Stages IVC and V [16]), deemed incurable by any means, by the multidisciplinary consultative head and neck clinic of the Mater Hospital, and suitable for palliative treatment only. Also included were four patients with lesser stage disease, but concurrent incurable second primary cancers arising from other sites (two from lung, one from pancreas, one from cervix). Ineligibility criteria included severe intercurrent illness likely to result in failure to comply with the treatment and follow-up monitoring laid down below. Also ineligible were patients who had received prior treatment for head and neck cancer, including previous radiotherapy or chemotherapy. Informed, written consent to participate was the final prerequisite for entry. The study protocol was approved by the Hunter Area Research Ethics Committee in September 1988.

2.2. Treatment details

Technical details of the project have been published elsewhere [5]. Briefly, a Siemens Caesium teletherapy unit has been adapted to treat at 80 cm SSD, using specially designed rectangular collimating cones providing field sizes of between 80 and 168 cm^2 (median size used 100 cm^2). Adjustments to instantaneous dose rate has been achieved using lead filters. Treatments have been conducted using large opposing equally weighted lateral beams, with dose and instantaneous dose rate specified at the midplane point on the central axes. All 51 patients treated at low dose rate received 10-week daily treatment fractions with both fields treated on each treatment day. Beam direction has been achieved through the use from front and back pointers and verified using portal films. Dosimetry based on water phantom depth dose measurements for each individual treatment cone, has been verified during treatment using entrance and exit thermoluminescent dosimetry on the central axes of the beam. All treatments have been conducted with the patients sitting or semi-reclining in a dental chair, modified for comfort with a customised headrest to minimise movement of the head. In addition to the monitoring of patient movement by the operator (SAS) on closed circuit television, a specifically designed optical proximity switch was used as a failsafe mechanism to temporarily suspend treatment if a predetermined degree of excessive movement had taken place. The operation of this switch is reported elsewhere [13]. Rest breaks were allowed at frequent intervals during each daily treatment because the shortest treatment was 1 h 4 min per day in the patient group treated to 32 Gy total dose at 3 Gy/h.

The 10 patients treated at high dose rate on this project were treated using opposing lateral 6 MV beams on a Varian Clinac 1800 linear accelerator. Once again, all patients had 10 daily fractions with both fields treated each day and dose specified at the midplane point on the central axes of the beams. All treatments were conducted with the patient supine.

Anti-fungal prophylaxis was not administered to any of the patients.

2.3. Study design

As previously described [5], a modification to the central composite design concept has been used in this project, with the aim of determining isoeffective doses for 10 daily fractions delivered at 0.8, 1.8, 3.0 and 240 Gy/h (high dose rate). The central point of the composite design was 38 Gy at 1.8 Gy/h. The dosing levels at each dose rate selected have been structured around isoeffect predictions, using the incomplete repair model [7,17], and separated by intervals of approximately 10% in total absorbed dose. This decision was based in part on the need to establish the sensitivity of the endpoints used [8], and ensure adequate sample size. For logistic reasons, allocation to each group was determined consecutively (as opposed to random assignment). The final dosing groups are described in Table 1.

2.4. Study endpoints

Oropharyngeal mucosal reactions were graded at least twice weekly during treatment and after, until full recov-

Table 1
Details of 'dosing' groups used in the project; total dose and instantaneous dose rate is specified at the midplane in the central axes of the beam

Dose rate (Gy/h)	Total dose (Gy)	No. of patients	No. of fractions	Overall time (days)
240	30	10	10	12–14
3	32	10	10	12–14
3	36	9	10	12–14
1.8	38	10	10	12–14
0.8	36	7	10	12–14
0.8	38	6	10	12–14
0.8	42	9	10	12–14

Table 2
Mucosal grading criteria used in FLDR study (EORTC/RTOG toxicity criteria)

Mucous membrane	
0	No change over base line
I	Injection; mild pain not requiring analgesics
II	Patchy mucositis which may produce an inflammatory serosanguinous discharge; may experience moderate pain requiring analgesics
III	Confluent fibrinous mucositis; may include severe pain requiring narcotics
IV	Ulceration, haemorrhage or necrosis

ery had taken place using the EORTC/RTOG acute reaction grading criteria (Table 2). Often observations were obtained more frequently to better estimate the day on which the reaction changed from one grade to another. Time in days, from the start of treatment, to the onset and recovery from each reaction grade was recorded for each patient in addition to the peak grade reached. This enabled calculation of the duration of time that each patient spent at each reaction grade. Also measured were qualitative skin reactions using a modification of the EORTC/RTOG acute reaction grading criteria (Table 2), and reflectance spectrophotometry skin readings and tumour response rates reported elsewhere [9,12].

2.5. Analytical methods

All data were entered onto a temporal database (MEDLOG). Non-parametric exact tests such as the Fisher, Wilcoxon and Kruskal-Wallis tests, were performed using the StatXact software package. Direct estimates of α/β and $t_{1/2}$ were derived using the α/βest program [2]. Log likelihood based 'goodness of fit' probabilities were derived using a custom designed spreadsheet program on Lotus 1-2-3, while FLDR reciprocal dose plots [19], and other curve-fitting procedures were carried out using the Sigmaplot software package. Reciprocal dose plots were derived using the method used by Scalliet et al. [19], and described by Dale et al. [6]. A dose rate correction factor 'K' is applied to each dose rate to derive an equivalent fractional dose 'd'. Full details are given in Scalliet et al. [19].

3. Results

3.1. Time course of reactions

Complete data sets (i.e., days to onset, recovery and duration of each reaction grade) for 56 of the 61 patients were collected. Death due to intercurrent causes in four patients, (one from myocardial infarction; one from cerebrovascular accident; and two from pneumonia), and haemorrhage from the primary tumour in one patient occurred after treatment but prior to full recovery of all mucosal reactions, and therefore prevented calculation of reduction durations at all grades in the remaining five patients. In Table 3, median times for each group of patients to reach and recover from each reaction grade are presented. It will be noted that median time to recovery from each reaction grade increases as total dose increases. Data concerning the duration of time spent at each reaction grade for each group, together with the proportions in each group that reach each reaction grade, are summarised in Table 4. These data confirm the impression gained from Table 3 that reaction duration increases with total dose. Strong linear relationships were found to exist between the duration of time spent at any one reaction grade and another.

3.2. Isoeffect conditions

ED_{50} (0.5 probability of reaction duration reaching a specific value) estimates at each dose rate, have been de-

Table 3
Median times in days (range in parentheses) to the onset and recovery from reactions at Grades I, II and III level (the values with asterisks are derived from data obtained from less than five patients)

Group	Onset						Recovery					
	Grade I		Grade II		Grade III		Grade III		Grade II		Grade I	
	median	(range)	median	(range)	median	(range)	median	(range)	median	(range)	median	(range)
30 Gy @ 240/h	7.5	(6–9)	12.5	(9–16)	16	(11–23)	20	(16–31)	23	(13–33)	26.5	(18–46)
32 Gy @ 3/h	8	(2–14)	13	(11–18)	17*	(15–17)	19*	(18–20)	21.5	(17–28)	25	(17–33)
36 Gy @ 3/h	11	(4–12)	13.5	(9–16)	16.5*	(14–18)	21*	(18–28)	26	(21–31)	27.5	(16–37)
38 Gy @ 1.8/h	6.5	(3–11)	10.5	(7–15)	15.5	(10–22)	22	(17–30)	24	(21–37)	27.5	(24–44)
36 Gy @ 0.8/h	10.5	(5–14)	15	(6–17)	15*	(8–17)	17*	(17–20)	23.5	(21–30)	26	(23–37)
38 Gy @ 0.8/h	10	(5–13)	14	(7–16)	17.5	(12–22)	20	(18–24)	22.5	(16–27)	27	(21–37)
42 Gy @ 0.8/h	8	(5–10)	12	(7–16)	15	(9–18)	21	(18–24)	26	(19–31)	31	(26–36)

Table 4
Median times in days spent at each reaction grade; proportions reaching each grade are also provided

Group	*n*	Duration of time spent at:									Proportions reaching:			ED_{50} (Gy)
		Grade I			Grade II			Grade III			Grade I	Grade II	Grade III	
		n	median	(range)	*n*	median	(range)	*n*	median	(range)				
30 Gy @ 240/h	10	10	19	(11–37)	10	12	(3–17)	10	3.5	(0–8)	10/10	10/10	7/10	30
32 Gy @ 3/h	10	10	16.5[1]	(7–28)	10	8[1]	(0–15)	10	0[1]	(0–5)	10/10	9/10	3/10[1]	35.5
36 Gy @ 3/h	9	8	19[2]	(9–30)	8	12.5[2]	(0–16)	9	4[2]	(0–10)	9/9	8/9	6/9[2]	
38 Gy @ 1.8/h	10	10	22.5	(17–33)	10	14	(11–24)	10	2	(0–10)	10/10	10/10	7/10	36.5
36 Gy @ 0.8/h	6	6	14.5	(9–29)	6	8.5	(5–18)	6	1	(0–9)	6/6	6/6	3/6	
38 Gy @ 0.8/h	7	6	20	(11–25)	6	11.5	(2–16)	6	2.5	(0–8)	7/7	7/7	6/7	39
42 Gy @ 0.8/h	9	6	25.5[4]	(16–30)	7	13[4]	(11–29)	9	7[4]	(0–9)	9/9	9/9	6/8[4]	
Composite group:														
37 Gy @ 0.8/h (= 36 + 38 Gy groups)	13	12	19[3]	(9–29)	12	10[3]	(2–18)	12	1.5[3]	(0–9)	13/13	13/13	9/13[3]	
Wilcoxon 1-S Exact *p* values			1 vs. 2	0.27		1 vs. 2	0.028		1 vs. 2	0.03	Fisher Exact *p* values	1 vs. 2	0.128	
			3 vs. 4	0.04		3 vs. 4	0.086		3 vs. 4	0.21		3 vs. 4	0.628	

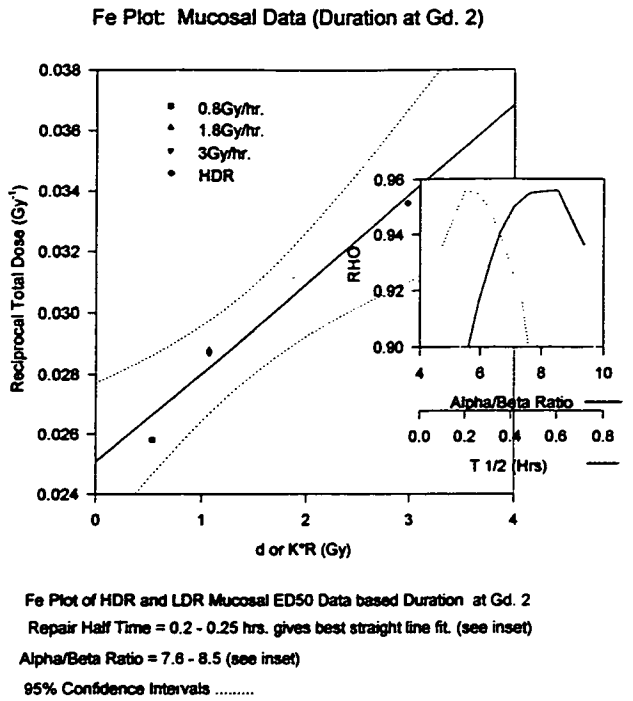

Fig. 1. Reciprocal dose plot for ED_{50} estimates for reaction durations at Grade II level. The inset graph describes correlation coefficient values (rho) for a straight line fit to the data points using various values of α/β and $t_{1/2}$. Best fit conditions are achieved by α/β values in the range 7.6–8.5 Gy and $t_{1/2}$ values in the range 0.2–0.25 h.

rived by interpolation from median and mean reaction durations at each reaction grade, as well as from the proportions in each treatment group whose reactions equal or exceed the median duration of reactions at each grade for the entire study population (Table 4). These estimates suggest that reactions to 30 Gy at 240 Gy/h are approximately equivalent to those caused by 35.5 Gy at 3 Gy/h (range 34.5–37 Gy), 36.5 Gy at 1.8 Gy/h (range 34.5–38 Gy), and 39 Gy at 0.8 Gy/h (range 38–40 Gy) at the three grades examined.

The ED_{50} estimates for Grade II reactions have been plotted on a modified reciprocal dose plot (Fig. 1). Best fit conditions are achieved by α/β values in the range 7.6–8.5 Gy, and $t_{1/2}$ in the range 0.2–0.25 h.

3.3. Direct quantal analysis, statistical uncertainty estimates

Direct quantal estimates of α/β and $t_{1/2}$, using the proportion of each group whose duration of reaction at each different reaction grade, equals or exceeds the median value obtained for the entire patient population combined, are provided in Table 5. Convergence on α/β estimates in the ranges α/β 6.7–10.4 Gy and $t_{1/2}$ 0.19–0.49 h are consistent with the results produced by the reciprocal dose plot methodology. It will be noted, however, that the 95% confidence intervals are quite wide, providing reason to explore the issue of statistical confidence further.

In Fig. 2, predictions of response proportions derived from the incomplete repair model are superimposed on the quantal responses actually observed in this project. It will be noted that while the α/β and $t_{1/2}$ parameters estimated for Grade II reactions (namely $\alpha/\beta = 8$, $t_{1/2} = 0.25$ h) provide a reasonable fit to the data, the parameters $\alpha/\beta = 15$, $t_{1/2} = 0.75$ h, provide a poor fit. In order to measure the goodness of fit of any given pair of parameter values to the observed data, the log likelihood ratio test for similarity between observed and expected binomial proportions was applied to a range of α/β and $t_{1/2}$ permutations, between α/β 5–15 Gy and $t_{1/2}$ 0.1–0.75 h. The results of this procedure for reactions at Grade II level are summarised in Fig. 3 and are in reasonable agreement with the direct analysis. This figure indicates that α/β values in the range 7.5–10 and $t_{1/2}$ in the range 0.2–0.55 h have a probability greater than 0.5 of fitting the data. Parameter values outside these ranges provide rapidly declining probabilities of fit, particularly for $t_{1/2}$ times below 0.2 h and α/β values above 15.

4. Discussion

An α/β estimate in the range 7–10 Gy, is entirely consistent with previous estimates [4,23]. The $t_{1/2}$ esti-

Table 5
Direct computations using the α/βest program of α/β and $t_{1/2}$ using the numerical proportion of patients in each group whose reaction durations equalled or exceeded the median duration of reactions at each reaction grade for the entire study population (the 95% CI for α/β and $t_{1/2}$ at Grades I and III are indefinite)

		α/β (95% CI)		$t_{1/2}$ (95% CI)	
Grade I	Logistic	10.3 Gy	(–)	0.18 h	(–)
	Poisson	10.4 Gy	(–)	0.19 h	(–)
Grade II	Logistic	9.0 Gy	(4.7–22.7)	0.24 h	(0.13–1.9)
	Poisson	9.3 Gy	(5.8–17.9)	0.25 h	(0.14–1.5)
Grade III	Logistic	6.6 Gy	(–)	0.43 h	(–)
	Poisson	6.7 Gy	(–)	0.49 h	(–)

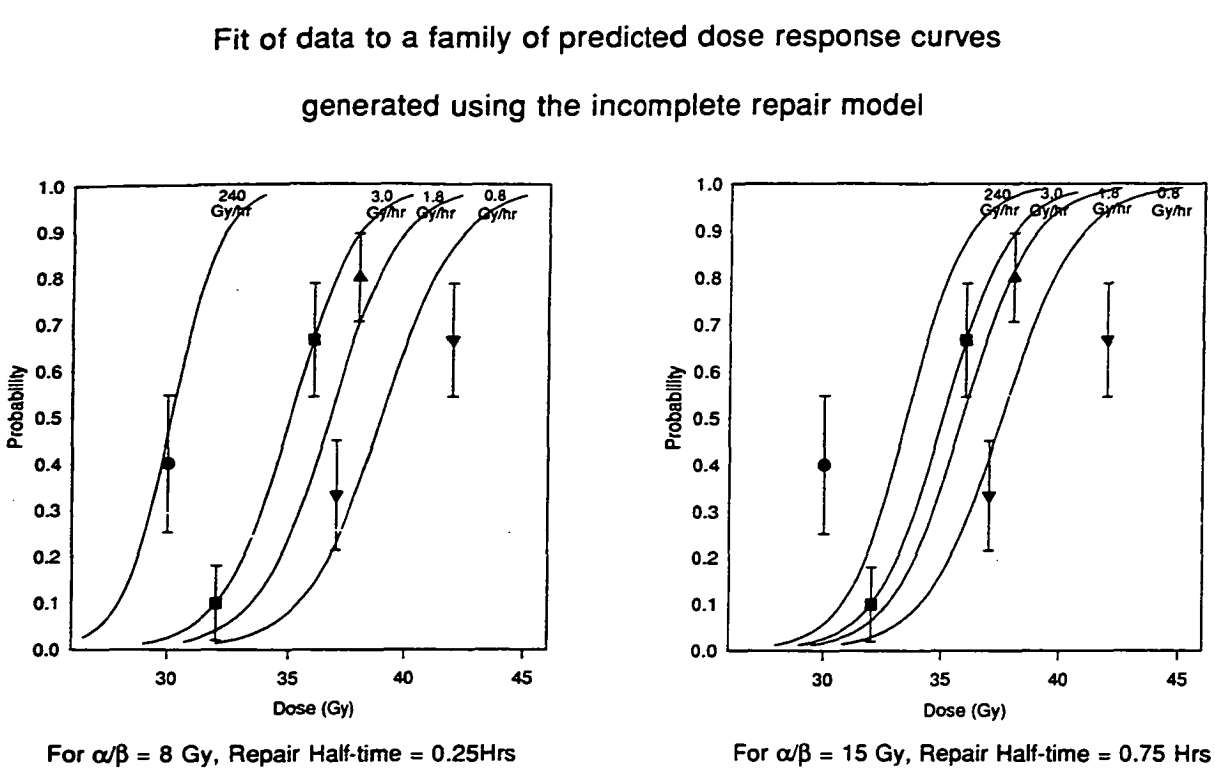

Fig. 2. Observed quantal responses superimposed on predicted response probability curves that were based on two different permutations of α/β and $t_{1/2}$ i.e., 8 Gy and 0.25 h and 15 Gy and 0.75 h. (N.B. The 'composite' group treated to 37 Gy at 0.8 Gy is formed from the groups treated to 36 and 38 Gy has been plotted.)

mate (range 0.25–0.5 h), however, is short and raises the question of the magnitude of uncertainties that may have impacted on the result.

These uncertainties can be addressed under three headings: technical aspects, the accuracy of the endpoints used, and sample size considerations.

4.1. Technical aspects

Turning first to the technical aspects it is clear that a number of factors could have impacted on dosimetric uncertainties. As discussed in an earlier report [5], the telecaesium beams used were not perfectly flat even at the 80-cm SSD used. Contour variations, scattering conditions and, above all, patient movement, provided additional uncertainties. Variation in overall treatment time is a factor that could have impacted on accumulated 'biological' dose, due to the possible onset of accelerated mucosal repopulation prior to treatment completion. Although it was intended the overall treatment duration would be 12 days, logistical difficulties prevented completion in less than 14 days in some patients. Although 'prolonged' treatments such as this did not affect any particular treatment group, it is a factor that may deserve revisitation as more becomes known of human mucosal repopulation kinetics.

4.2. Endpoints

Concerning the accuracy of the endpoints used, we have already drawn attention in a previous report to the fact that it is far easier to determine the level of a reaction on any particular examination day, than to estimate precisely how long a reaction at any particular grade lasts [8]. Factors involved include the matter of judgement that a reaction is changing from one grade to another on any particular day. Making this judgement more difficult is the fact that subsiding reaction appearances can be different to reaction appearances that are still evolving. Anatomical site differences, the presence of tumefactions and residual dentition are additional problems that we attempted to avoid by concentrating on the appearance of the oropharyngeal mucosa. Of the three reaction grade duration endpoints used in this study, we feel that duration at grade II has probably produced the most reliable α/β and $t_{1/2}$ estimates. Duration at the Grade I endpoint (erythema) is a difficult measure to judge reproducibly, and may not reflect a purely cytological endpoint [9]. Estimates based on duration at Grade III may be less reliable because only 37 of the 61 patients experienced this grade.

4.3. Sample size

Finally, the issue of sample size, which is one of the most important considerations in any study, is clearly an important one in this project. Uncertainties due to sample size perhaps dwarf other sources of error already mentioned. The wide confidence intervals around the re-

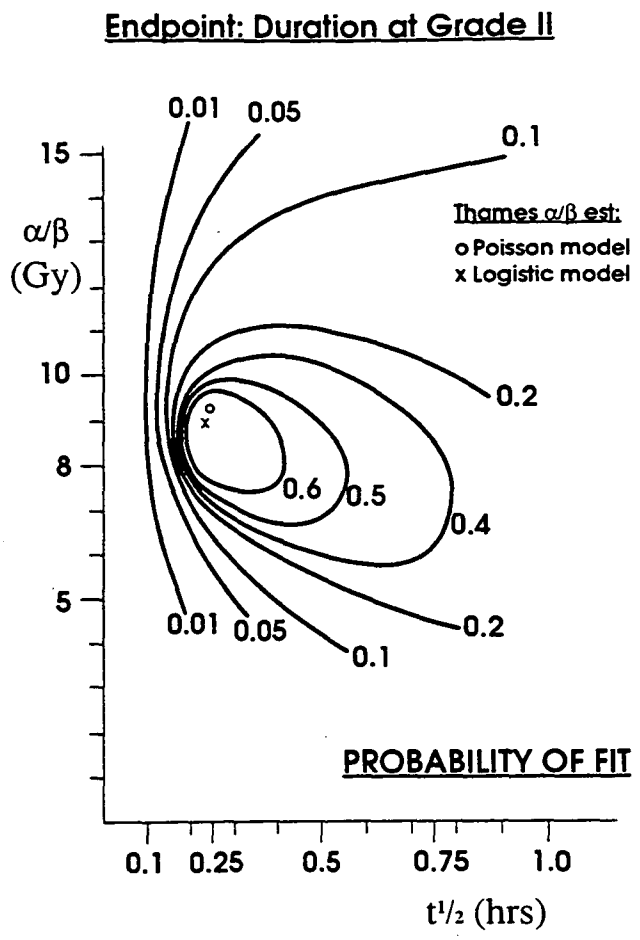

Fig. 3. A matrix of probability values that the observed data will fit various permutations of α/β and $t_{1/2}$ specified on the axes.

sults estimated by direct analysis (Table 5), and the diagram referring to goodness of fit to the data of a range of permutations of α/β and $t_{1/2}$ (Fig. 3), demonstrate this uncertainty quite clearly. Of course, these uncertainties are entirely due to the small numerical size of each treatment group. It is difficult to know whether a doubling of the sample size would have significantly increased the precision of our estimate, but this was not an option due to logistical and ethical constraints. While the logistical constraints of doing this are obvious, the ethical arguments are less straightforward. In any human volunteer study in which therapeutic benefits from the test treatment are not expected to exceed the benefit to be obtained from 'conventional' treatment [12] (i.e., fractionated palliative radiotherapy or chemotherapy), the ethical imperative is to discontinue the study after treatment of the minimum number of patients likely to produce a plausible result from the study. After we had become satisfied that a minimum of nine patients in each treatment group would allow us to resolve a difference in total physical dose of 10% [8] in our test system with all its uncertainties, we took the decision that the result obtained using groups of the stated size would satisfy these minimum conditions, i.e., be plausible.

Few published values for the α/β and $t_{1/2}$ values for mucosa are available for comparison with our results. Chougule and Supe recently reported α/β ratios in the range 7.68–8.11 Gy for acute mucosal reactions in patients with head and neck cancer treated with twice daily fractionation schedules [4]. Acute reactions in the mouse lip are one of the 'nearest' laboratory models. Various estimates of α/β have been obtained using this system. Ang et al. derived a value of 8.5 Gy using large fractional doses at high dose rate [1]. In a study employing a range of continuous low dose rate treatments, delivered at various dose rates, Scalliet et al. derived a value of 7.4 Gy [19]. In a subsequent study which investigated a range of interfraction intervals using fractionated high and low dose rate irradiations, Stuben et al. derived values in the 14.1–18.2 range [21]. Values for repair $t_{1/2}$ were in the 0.5–0.75 h range in the two sets of experiments that employed low dose rate irradiation [19,21], but were higher (0.9–1.2 h range) in the fractionated high dose rate study conducted by Ang et al. [1]. In mouse tongue mucosa, Dörr et al. derived an α/β ratio of 11.6 Gy and a $t_{1/2}$ value of 0.75 h in a study that involved high dose rate irradiation at various interfraction intervals [11]. Thames et al. concluded that human tissue repair $t_{1/2}$ values are usually slightly longer than those obtained in rodents [23]. However, the only value for the human mucosa published to date is 2 h or more, which grossly exceeds the mouse lip estimates [23]. It also exceeds the direct analysis estimates of 1.3 h for erythema and 1.1 h for desquamation in human skin obtained from Turesson and Notter's internal mammary irradiation data [26,27]. The 2-h estimate for the human mucosa was derived from data obtained in the randomised RTOG trial in advanced head and neck cancer, which compared a conventionally fractionated treatment of 66–74 Gy in 7–8 weeks, with the 60 Gy in 5 weeks utilising twice daily fractions of 1.2 Gy reported by Marcial et al. [15]. In this study interfraction interval ranged from 3 to 7 h. Both early and late reactions occurred more frequently in patients whose interfraction intervals were less than 4.5 h. 'Severe mucositis' occurred in 20 of 72 (28%) patients treated with interfraction intervals less than 4.5 h, whereas it occurred in only one of 20 (5%) of patients treated with longer intervals ($p = 0.06$). Since this was a multi-institution study, in which patients were not randomly assigned to specific interfraction intervals and treatment was interrupted in 36% of patients treated on the twice a day schedule, it is difficult to know what significance to attach to these observations. However, increased mucositis was also reported in a pilot study of concomitant boost therapy in Poland [3]. In this study the incidence of confluent mucositis during the concomitant boost phase of treat-

ment was reduced when interfraction interval was increased from 4 to 6 h. Steel et al. noted that in cell line experiments shorter $t_{1/2}$ estimates were obtained from dose rate analyses employing a range of low and intermediate dose rates, than from split dose experiments using high dose rate irradiation [20]. Dale et al. witnessed a similar phenomenon for dose rates up to 25 Gy/h in the C3H mouse jejunum [7], which added weight to the suggestion that repair may be a multi-component process that is not always well described by a mono-exponential function. Perhaps this explanation provides some reconciliation between the long estimate obtained from the RTOG and Polish mucosal data, and the short estimate obtained in this project. It could be that repair of a rapid component of what is in fact a multi-exponential repair process in human mucosa is insignificant after irradiation at high dose rate, but is important during protracted exposure at low dose rate. If this is true then the incomplete repair variant of the linear quadratic model, which assumes mono-exponential repair kinetics, does not provide a perfect description of acute mucosal reactions under all conditions as it stands. It would also mean that parameters derived from dose rate studies, such as this one, should be applied with extreme caution to fractionated treatment courses delivered at high dose rates.

5. Conclusions

This project has produced data that provide α/β ratios of 7–10 Gy and $t_{1/2}$ values of 0.25–0.5 h for the acutely reacting human mucosa when the incomplete repair model is applied. The $t_{1/2}$ values are short, however, and raise the question as to whether the repair kinetics of this tissue are well described by a mono-exponential function. Further prospective studies involving multiple daily fraction treatment regimes delivered at high dose rate, in which interfraction interval is deliberately varied, are needed to know whether the parameters derived from this project are applicable to fractionated treatment courses at high dose rate.

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Factors influencing the degree of erythematous skin reactions in humans

J.W. Denham*[a], C.S. Hamilton[a], S.A. Simpson[a], P.M. Ostwald[a], M. O'Brien[a], T. Kron[a], D.J. Joseph[a], K.B.G. Dear[b]

[a]*Department of Radiation Oncology, Mater Misericordiae Hospital, Waratah, NSW 2298, Australia*
[b]*Statistics Department, The University of Newcastle, Callaghan, NSW 2308, Australia*

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Abstract

Dose-response relationships have been studied using an ordinal visual scale and reflectance spectrophotometry data from 123 treatment sites on 110 patients treated with 10 dose fractions over 12–14 days. Dose rates varied between 3 and 240 Gy/h and total doses of between 25 and 41 Gy were given using teletherapy apparatus. We found qualitative scoring of erythematous skin reactions to be subject to considerable inter- and intra-observer variation. Reflectance spectrophotometry provided more reproducible information, some of which was undetectable by naked eye. Baseline erythema readings were significantly higher in male patients and at anatomical sites of previous heavy UV exposure. In addition, a pronounced decline in erythema readings during the second week of therapy and 'reciprocal vicinity' (abscopal) effects adjacent to the field, undetected by the eye, were observed in a subset of patients. Meaningful dose-response relationships could be derived only from reflectance data with peak change from the pretreatment baseline measure providing the best discrimination. Peak erythema measures following treatment were found to depend on the age and gender of the patient as well as the treatment site and its baseline erythema measurement. This was independent of the total dose administered or the instantaneous dose rate at which it was delivered. The rate of erythema development was also dose rate dependent but only weakly dependent on the biological dose intensity (Gy equiv./day) of the treatment course. The data raise the question of whether irradiation-induced erythema is exclusively a secondary phenomenon occurring as a result of basal cell killing. The short repair half time value of 0.06 h obtained by direct analysis is perplexing and may reflect a dose rate-dependent physiological vasodilatory response to irradiation and/or a multi-component cellular repair process.

Keywords: Skin; Erythema; Radiotherapy; Dose rate effect; Reflectance spectrophotometry; α/β ratio; Repair kinetics

1. Introduction

The mammalian integument has proven a fertile area for radiobiological research. In particular, observations on the mouse and pig have been responsible for much of the data which has helped to formulate modern concepts of fractionation, dose rate effects and field size effects [11,14,17,22,24,25,34,53]. Its accessibility both to the delivery of ionising radiation and observation, coupled with its non-vital role for laboratory survival in most small mammals, has contributed also to its wide applicability in primary radiobiological research. Laboratory workers have used a variety of ordinal scoring scales for evaluating acute skin reactions. Similar, but usually simpler, scoring systems for humans have been derived over the past several years. These include the RTOG and EORTC scales and the Dische inventory [13]. These scales endeavour, as much as possible, to simplify and standardise observations which have been noted in clinical radiotherapy for many years. Most human radiobiological data has derived from the use of ordinal scales such as those described above [41,50]. However, these scales are subject to considerable inter- and intra-observer variation. Reflectance spectrophotometry is a quantitative technique which has been employed by various workers [33,38,44–48] in an effort to overcome these problems. The most extensive experi-

* Corresponding author.

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ence with the technique of reflectance spectrophotometry, for the assessment of radiation reactions in humans, comes from the work of Turesson and colleagues [43,44,49], concerning the acute skin reactions in the treatment of bilateral internal mammary node chains in women treated post-operatively for breast cancer. Turesson demonstrated that reproducible quantitative evaluation of skin reactions was possible, at least up to the level of and including dry desquamation. Nias [33] utilised reflectance spectrophotometry in some thirteen patients and was able to demonstrate a dose response and fractionation effect, although he took these studies no further. Russell et al. [38] also utilised reflectance spectrophotometry to assess skin erythema response to post-mastectomy radiotherapy, but were unable to demonstrate a significant dose-response relationship.

It is known that the overlapping absorption spectra of the main pigments that influence skin colour (deoxy-haemoglobin, oxy-haemoglobin and melanin) can result in incorrect haemoglobin (hence 'erythema') readouts. This is of potential importance in clinical radiobiological studies because increases in erythema are paralleled by increases in melanin during conventionally fractionated courses of radiation. The development by Feather, Dawson and colleagues of a unit [10,20,21,23] that overcomes the problem of the interdependence of the skin pigments on the 'erythema' readout was therefore of interest and was used in a series of clinical radiobiological studies that commenced in 1989.

One of these studies involved the use of this unit to assess skin erythema in investigating the effects of fractionated 'low' (medium) dose rate (FLDR) radiotherapy. This study was designed to identify dose rate, fractionation (α/β ratio) and repair ($t_{1/2}$) parameters for cutaneous erythema in response to FLDR treatment. With a large data set of matched qualitative and quantitative scores, we therefore had the opportunity to perform a detailed comparison of these methodologies and investigate the influence of various factors (e.g., sex, sun-exposure and treatment site).

It quickly became apparent that the reflectance spectrophotometry measures that we were using to assess skin reactions in patients were 'noisier' than measures obtained in previous studies which had been confined to one anatomical site in patients of one gender [44,45]. Our patients had been treated palliatively for metastatic disease which meant that a variety of anatomical sites, subject to varying degrees of prior sun exposure had been treated. The patients themselves varied in age and sex. We decided, therefore, to investigate the causes of variation in reflectance measures obtained in our data set to find out whether the factors identified might be responsible for variations in the parameters that influence dose response. The results of this investigation are therefore also presented in this report.

2. Materials and methods

2.1. Patients

The Department of Radiation Oncology at the Newcastle Mater Misericordiae Hospital, embarked on an experimental fractionated low dose rate (FLDR) teletherapy project in 1989 [7] following approval by the Hunter Area Health Service Regional Ethics Committee. This project was designed with the aim of assessing acute normal tissue end-points (mucosa and skin) in an effort to provide iso-effect data for a range of total doses and dose rates. This report refers to data from 91 patients irradiated with 104 individual fields at either 3.0, 4.8 or 8.2 Gy/h on a Siemens Caesium-137 teletherapy unit and a further 19 patients treated at high dose rates on a Varian Clinac 1800 at 6 MV, using identical selection criteria. Consenting patients comprised those with a variety of metastatic solid tumours with systemic disease in relatively superficial sites, e.g., lymph nodes or axial skeleton. Selection criteria are given in Table 1.

Due to logistical constraints patients were accrued sequentially, rather than by random assignment, to treatment according to a central composite design of total dose and dose rate points based on the predictions of the incomplete repair model applied at medium and low dose rates [8]. Treatment allocation was not prospectively stratified according to age, gender or other potential explanatory variable and the resulting case composition of the series grouped according to treatment dose rate is provided in Table 2.

All patients accrued on to the study were of Caucasian extraction. Most were of Celtic and Anglo-Saxon descendancy. The remainder had middle Mediterranean

Table 1
Patient selection criteria

	Bony and soft tissue metastases
(1)	Proven symptomatic metastatic cancer involving sites that can be encompassed satisfactorily by modified caesium beam at 60 or 80 cm SSD
(2)	A disease site that has not been previously irradiated, is not situated in a pressure area and in which there is no evidence of skin or subcutaneous involvement by tumour.
(3)	The patient has not received adriamycin, actinomycin D, or bleomycin containing regimes, or is being concurrently treated with cytotoxic chemotherapy or other agents that may modify radiation response.
(4)	Ability to lie still for a period of up to 1 hour
(5)	Written informed consent for treatment on protocol.

Table 2
Case composition of the study population according to dose rate grouping

	Dose rate groups			
	3 Gy/h	4.8 Gy/h	8.2 Gy/h	High dose rate
Total number of fields	17	43	44	19
Age, years; median (range)	66 (41–90)	70 (43–82)	71 (28–88)	64 (46–84)
Male, no. (%)	8 (47)	29 (67)	34 (77)	15 (79)
Heavily sun-exposed fields, no. (%)	6 (35)	26 (60)	30 (68)	8 (42)
Head and neck	7	14	15	1
Upper torso	0	10	14	7
Lower torso	4	7	4	8
Pelvis	4	6	5	3
Limbs	2	6	6	0
Total measured dose, Gy; median (range)	36.1 (34.5–41.4)	36.95 (32.8–41.2)	36.9 (31.3–40.6)	31.05 (25.1–34.2)
Overall time, days; median (range)	14 (12–16)	14 (12–15)	14 (12–18)	15 (12–20)
Field size, cm^2; median (range)	101 (72–273)	92 (57–273)	92 (57–168)	144 (66–400)
Full set of reflectance measures, no. of fields	15	27	28	19

backgrounds. Solar skin damage, of a severity not usually seen in Europe and the United States, was a common occurrence in the study population and the number of treatment fields affected by heavy prior sun exposed are also detailed in Table 2.

2.2. Irradiation technique

Teletherapy at low and medium dose rates was delivered using a modified Caesium-137 teletherapy unit at extended SSD (60–80 cm). Patients were treated using direct appositional fields to cover relevant bony or soft tissue disease. Tissue equivalent bolus (2 mm) covered the entire field during treatment and dose prescription was to the point of maximum electronic build up (D_{max}) which for the Caesium unit used is 1.0 mm below the treatment surface. Specially designed low melting point alloy collimator cones were constructed to define the beam and minimise penumbra. By the use of appropriate lead attenuation filters, instantaneous incident dose rates of 3, 4.8 and 8.2 Gy/h were achieved. Fraction times varied between 40 and 90 min per day and careful consideration was given to patient comfort and immobilisation during treatment. Treatment was administered daily (ten fractions) over a total time period of 12–14 days and optical proximity switches were used to confirm patient position and interrupt treatment if significant movement occurred [26]. Patients receiving high dose rate teletherapy were treated using single 6-MV fields to an identical range of body sites for a variety of soft tissue and bony metastatic disease. Treatment was delivered on a Varian Clinac 1800, with dose prescribed to D_{max} which is 15 mm below the treatment surface. Treatment was administered daily (ten fractions) over a total time period of 12–14 days. If patient geometry and field size permitted, tissue equivalent bolus (15 mm) was used to cover half the field for each treatment.

2.3. In vivo dosimetry

To derive estimates of dose relevant to the basal cell layer of the skin, skin dose measurements for 'low' dose rate treatments on the Caesium unit were performed using paired conventional lithium fluoride thermoluminescent dosimeters (TLDs) (diameter 4.5 mm, thickness 0.8 mm) (Vinten TLD100) which were applied to the field centre on each patient under 2 mm of dental wax build up on the first day of the treatment course. Results were averaged and if the two readings differed by more than 10%, the results were discarded and the readings repeated. For high dose rate treatments at 6 MV, paired carbon-loaded TLDs (Vinten) [42] were inserted into a tissue equivalent polystyrene holder and applied to the field centre on the patient's skin. No additional build up was utilised for these measurements. Where 15 mm of bolus was present over half the field, a pair of conventional lithium fluoride (LiF) TLDs were placed beneath the bolus. In all dose-response analyses subsequently described in this report, measured skin dose rather than prescribed dose has been used.

2.4. Qualitative erythema scoring

All patients on treatment were evaluated twice weekly by two independent observers, with scored skin reactions according to a modified EORTC/RTOG scale (Table 3). Following treatment skin reactions were evaluated at least weekly until all signs of reaction had subsided.

2.5. Quantitative erythema measurement

Reflectance unit

Reflectance spectrophotometry measurements were performed using an instrument (Spectral Research Pty Ltd, Leeds, UK) developed by Feather et al. [10,20,21,23]. Thirty-four fields studied prior to the unit's arrival in 1991, and included in the present analysis, did not have a full set of reflectance readings (Table 2). Full details on construction and validation of the performance of the unit have been provided by Feather

Table 3
Modified EORTC/RTOG grading system for acute skin reactions used in this project

Skin
0 No change over base line
1A[a] Follicular: faint erythema, epilation, dry desquamation, decreased sweating
1B[a] As above but confluent bright pink erythema
2 Tender or dusky erythema, patchy moist desquamation, moderate oedema
3 Confluent, moist desquamation other than skin folds, pitting oedema
4 Ulceration, haemorrhage, necrosis

[a]This distinction is a local one and therefore represents a modification.

and colleagues [21,23]. Briefly, the unit samples at 150 different wavelengths throughout the entire optical spectrum (400–700 nm) to derive estimates of deoxy-haemoglobin, oxy-haemoglobin and melanin 'content'. Software incorporated within the unit corrects the reflected values measured for the influence of absorption by each pigment on the others prior to output. It achieves this by evaluating the gradients of the log inverse reflectance spectrum between isobestic points for the deoxy- and oxy-haemoglobin absorption spectra at 527.5, 544 and 573 nm to provide a haemoglobin index independent of the oxygenation of the haemoglobin and skin melanin content. A combination of this index with measurements at 558.5 nm then provides a measure of oxygen saturation [21]. Finally, evaluation of the gradients of measurements obtained at 650 and 700 nm provide a measure of melanin pigmentation that is independent of both deoxy- and oxy-haemoglobin [23]. If desired, all 150 reflected values across the entire optical spectrum can be read out.

Reflectance readings were taken pre-treatment and at twice weekly intervals during and at least weekly after the course of radiotherapy, until the acute erythematous reaction had returned to baseline. Measurements were taken in the recumbent position, after 5 min rest in a constant ambient temperature of 22°C. Five measurements of the haemoglobin index were obtained at the centre of the treatment field and the mean of these used to represent the reflectance reading for that day. In a similar fashion, five readings were also taken at a 'control' point at least 3 cm outside the radiotherapy treatment field. The measurement constancy of the reflectance unit was checked on a daily basis utilising a specific artificial absorbence filter (Wratten filter) (mean daily reflectance reading 53.33 ± S.D. 2.23).

Pilot reflectance study

In order to test the usefulness of baseline reflectance readings and assess the impact of physiological variables, treatment related variables unconnected with X ray dose and previous sun-exposure, a control study wa designed in which twelve patients (six males, six fe males), who were undergoing a course of conventionall fractionated external beam radiotherapy, had twic weekly reflectance readings performed at sites distant t their irradiated field. Three of each of the six patients i each group had measurement sites chosen which had nc been previously sun-exposed and the remaining thre had sun-exposed measurement sites chosen. In order t assess the temporal variability of control readings, a se quence of five erythema indices were measured and re peated at intervals of 8 min over a 40-min perioc During this 40-min measurement period, the pulse rat blood pressure (systolic and diastolic), respiratory rat and skin temperature (Medtel instruments), on each c the twelve control patients was measured. An average c 62 measures per patient was obtained.

2.6. Analytical methodology

All data was transcribed onto a PC-based tempora database (MEDLOG, Systemedica), and simple descrip tive statistics and quantal response data, based on th erythema measures exceeding given values, wer generated directly from this database. The generalise linear regression model (type III Statistical Analysis Sys tems) was used to correlate observed reaction grade with reflectance data and to test for the independent e fects of age, sex, site, sun-exposure, measured dose an instantaneous dose rate on the dependent variable baseline erythema measure, rate of erythema develop ment and the various peak erythema measures testec These peak measures included:

(1) Raw peak erythema (E_p). Erythema at time t is E

(2) Peak change in erythema (peak erythema minu initial erythema $E_p - E_0$). This measure represents a *absolute* change in erythema value.

(3) Peak erythema index (peak erythema divided b initial erythema E_p/E_0). This measure represents a *pro portional* change in erythema measure. Erythema inde at time t is E_t/E_0.

(4) Peak percentage change in erythema (pea erythema minus initial erythema divided by initia erythema, all multiplied by 100)

$$\text{i.e., } = \frac{E_p - E_0}{E_0} \times 100$$

This method also represents a proportional change i erythema measure.

(5) Duration of reaction at 6 erythema units abov baseline.

(6) Duration of reaction at 12 erythema units abov baseline.

(7) Duration of erythema at 18 units above baseline.
(8) Area under the total reaction curve.

The coefficient of variation for inter and intra patient variation was calculated using

$$S.D./\bar{x} \times 100\%$$

where S.D. is the standard deviation of all sequential control erythema values for each individual patient (intra-patient variation) and $\bar{x}$ is the mean of each patient's sequential control erythema values. For inter-patient variation, S.D. is the standard deviation of all sequential control erythema readings for the group or sub group under consideration and $\bar{x}$ is the mean for that group or sub-group.

2.7. LQ analytic techniques

A modification of the reciprocal dose plot methodology [14] was used to provide an indirect estimate of the α/β ratio and the half time of repair $t_{1/2}$. This method requires conversion of the 'low' dose rate data was required to be converted to an 'effective' dose per fraction (d). Dale [8] defined a new parameter K which, when multiplied by the dose rate, is analogous to the dose per fraction (d) in fractionated high dose rate radiation. K depends upon the parameter μ (the repair constant, units h^{-1}) which in the analyses presented herein was varied to obtain the most plausible fit of different permutations of α/β and $t_{1/2}$ values to the data.

Direct estimates of the α/β ratio and repair half time were obtained using the $\alpha\beta$est program of Bentzen et al. [4]. This program uses individual patient data to derive estimates. For the purposes of these analyses, a "response" in the individual patient was defined as a peak erythema measure exceeding the median peak erythema value for the entire study population.

3. Results

3.1. Patient variation

The mean control reflectance values and the coefficients of variation at an inter- and intra-patient level for the study patients and the twelve pilot patients are presented in Table 4. Both these groups of patients clearly demonstrated differences in control reflectance readings, related to both gender and previous sun-exposure of the site under consideration (for FLDR patients this control reading was at least 3 cm outside the treatment field and for pilot patients these readings were derived from another body region). Multiple linear regression analysis for the FLDR patients in the pilot study, using the same explanatory variables, demonstrated identical trends and significance values in the two sets of patients. Males with sun-exposed measurement sites had higher baseline reflectance readings in both the FLDR study and pilot study patients. The study control sites tended to show higher coefficients of variation at an inter-patient level in comparison to the pilot study patients. Multiple linear regression analyses on the FLDR study patients (not shown) demonstrated that both the baseline and entire set of out of field control erythema readings were significantly correlated with both gender and site. Although sun-exposure was strongly correlated, when considered as a solitary variable it failed to maintain significance in the multiple regression procedures when measurement sites in the head, neck and upper torso, which themselves were frequently heavily sun exposed, were taken into account. These sites were associated with higher control erythema readings than other less exposed sites such as the lower torso and pelvis. Additional findings included an association between the baseline erythema measure and melanin pigmentation.

Both the FLDR study and pilot study patient groups demonstrated similar intra-patient variability with mean coefficients of variation of 12 and 11.9%, respectively. The 12 cancer control patients exhibited no systematic variation in erythema values over the 4–6-week measurement period nor during the post-treatment measurement period (40 min). No significant linear or non-linear trends were seen with measured haemoglobin values and any of the physiological parameters studied (blood pressure, pulse rate, respiration and skin temperature).

Table 4
Coefficients of inter- and intra-patient variation for the study group and the pilot group

	Study group (n = 89 control sites)		Pilot (n = 12)	
	Mean value	Coefficient of variation	(%) Mean value	Coefficient of variation (%)
Inter-patient variation				
Males				
Sun-exposed	20.6 (n = 37)	38.9	25 (n = 3)	22.3
Non-exposed	14.2 (n = 22)	30.3	13.1 (n = 3)	14.5
Females				
sun-exposed	17.2 (n = 13)	28.9	17.5 (n = 3)	8.6
Non-exposed	14.5 (n = 17)	31.7	8.1 (n = 3)	29.7
All	17.4	39.8	16.3	41
Intra-patient variation	mean coefficient of variation 12%		mean coefficient of variation 11.9%	
	range	4–30%	range	5–24%

The study group had an average of 11.8 sequential readings per patient and the pilot group an average of 62.

3.2. Time course of observed reactions

The time course of graded skin reactions and reflectance measures following the FLDR regimes applied in this study showed several typical patterns. The reaction peak was seen between 30 and 40 days from the commencement of treatment and had returned to baseline between 90 and 110 days. It will be noted in Fig. 1 that the rate at which erythema developed was more rapid in patients treated at high dose rate. In 46% of cases, observed graded skin reaction corresponded temporally with the measured erythema index (Fig. 2A). In 34% of cases a rise in erythema index was clearly seen to precede the observable subjective response (Fig. 2B). In 7% of cases the observed graded skin reaction clearly preceded the rise in measured erythema index, and in a further 4% the reflectance score both preceded the rise in observed skin reaction and return to baseline well after the observed skin reaction had settled. In a small percentage of patients (9%) no discernible correlation existed. In approximately one third of cases, a clear 'dip' in the measured erythema reading, usually seen in the second week of treatment, was demonstrated. This 'dip' was not detected in the qualitative visual scoring (Fig. 2B) and sometimes descended below baseline levels. In 23% of cases the measured control reflectance readings, measured simultaneously 3 cm outside the treated field, appeared to parallel the in-field responses (Fig. 2C). These temporal patterns in and out of the field were not related to gender, age, site or sun-exposure, but did appear to be influenced by the dose rate at which treatment was delivered. Out of field reactions were more frequent and more intense in patients treated at high dose rate.

3.3. Relationship of reflectance reading to observed skin reaction grade

For identical reflectance erythema readings, male patients, melanin pigmented and sun-exposed anatomical

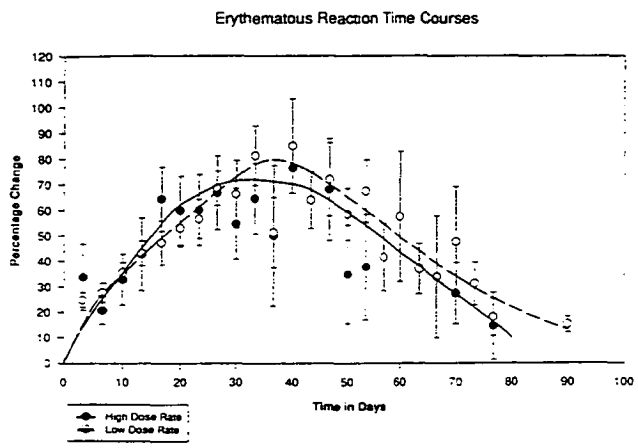

Fig. 1. Pooled time course data for patients treated at high dose rate and for the entire group of patients treated at 'low' dose rates.

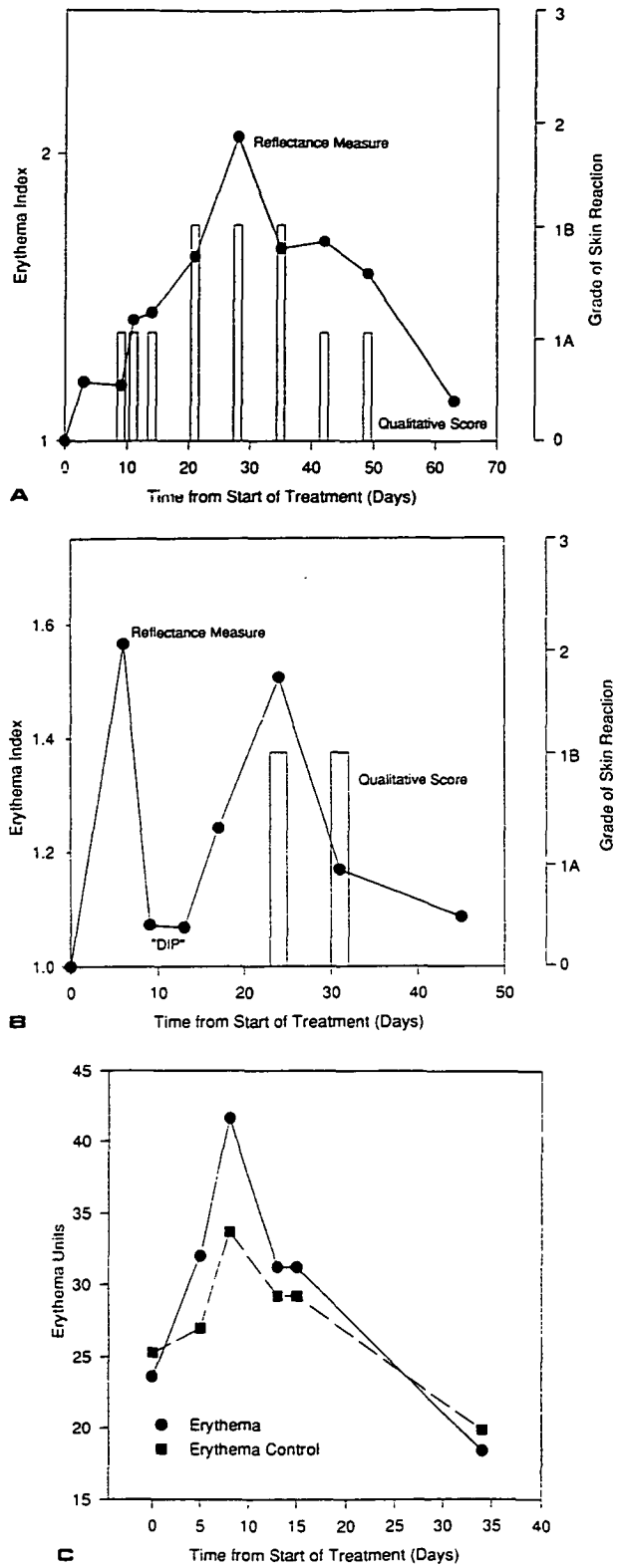

Fig. 2. (A) Typical patient reaction curve demonstrating close matching of measured erythema index (closed circles) and observed skin reaction grade (open bars) with time. (B) Typical patient reaction plot demonstrating clear rise in erythema index (E_i/E_0) (closed circles) prior to observed erythema and existence of a dip in erythema measure during the second week of therapy. (C) Suggestive temporal relationship of in-field and control erythema readings in a typical patient demonstrating this phenomenon.

sites were assigned lower visual reaction grades. Division of the erythema measure by the baseline measure (i.e. E_t/E_0) or subtraction (i.e., $E_t - E_0$) reduced the size of these variations and produced a substantial improvement in the correlation between observed grade and reflectance measure. The relationship between erythema index and graded score did not vary substantially during the time course of the reaction, with similar correlation coefficients, slopes and intercepts observed in the early, middle and late parts of the reaction.

Whilst reasonable correlation was found to exist between reflectance values and the observed skin reaction score, good concordance was not (Fig. 3) with substantial overlap in grade assignment. For differences of 15 units between the observed reading and the baseline reading, some 10% of assigned ordinal scores were zero, 48% of assigned scores were graded at 1A and 30% at 1B. It was only below differences of 5 units that there was good consensus that no reaction (zero grade) was present. It was only above differences of 20 units that perfect consensus existed that a reaction was present, but there was no consensus as to its grade.

3.4. Factors influencing initial erythema value

Initial reflectance values were found to vary from individual to individual even prior to the application of treatment. Results from generalised linear regression analysis (not shown) suggested that male patients and head and neck sites returned the highest baseline erythema values, independent of other variables. In addition, higher baseline erythema readings were seen to correspond with higher baseline melanin readings.

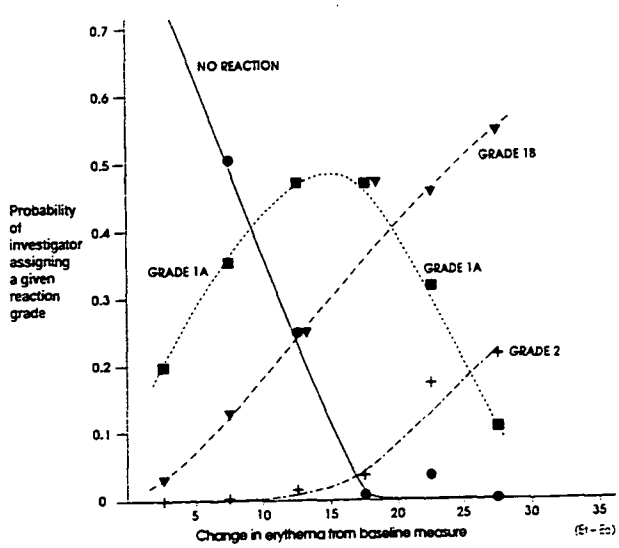

Fig. 3. Probability of observer assigning a given reaction grade for different changes in erythema measure from the baseline measurement ($E_t - E_0$). The data points represent data binned at 5-unit intervals.

3.5. Dose-response relationships

'Peak erythema' measures (E_p) failed to correlate with measured surface doses. In some patient subsets, small positive relationships were observed but in some, small negative relationships were noted and, in all, correlation coefficients were low. The 'peak erythema index' (E_p/E_0) and 'peak percentage change' ($[E_p - E_0]/E_0 \times 100$), on the other hand, produced positive correlations with increasing measured dose in all patient subsets examined, with correlation coefficients that ranged between 0.12 and 0.32 but none that differed significantly (statistically) from zero. Area under the reaction curve and duration of reaction at 6 units above baseline also produced positive dose-response correlations in all patient subsets but with even less significant correlation coefficients. Inferior correlations were seen for duration of reactions at 12 and 18 units above baseline due to an increasing number of zero scores in all subsets. No significant differences in dose response between groups treated at 3, 4.8 and 8.2 Gy/h were observed (Fig. 4), but a difference between patients treated at high dose rate and *all* patients treated at low dose rates was seen and is depicted in the quantal response plots presented in Fig. 5.

Fig. 5 also demonstrates that *no* dose-response relationship could be elicited for duration of time spent at Grade 1A or 1B observed reaction grades. Unfortunately no dose-response relationship could be elicited from the proportion of patients reaching each reaction grade either.

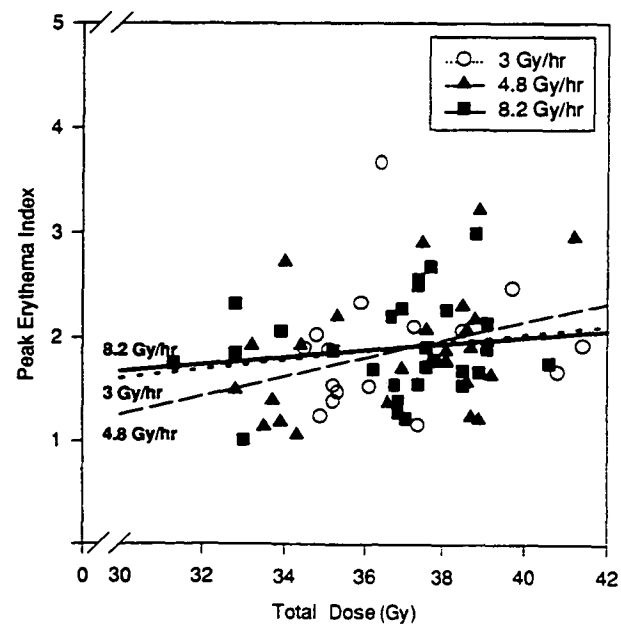

Fig. 4. Peak erythema index (E_p/E_0) according to total measured dose for fields treated at 3, 4.8 and 8.2 Gy/h.

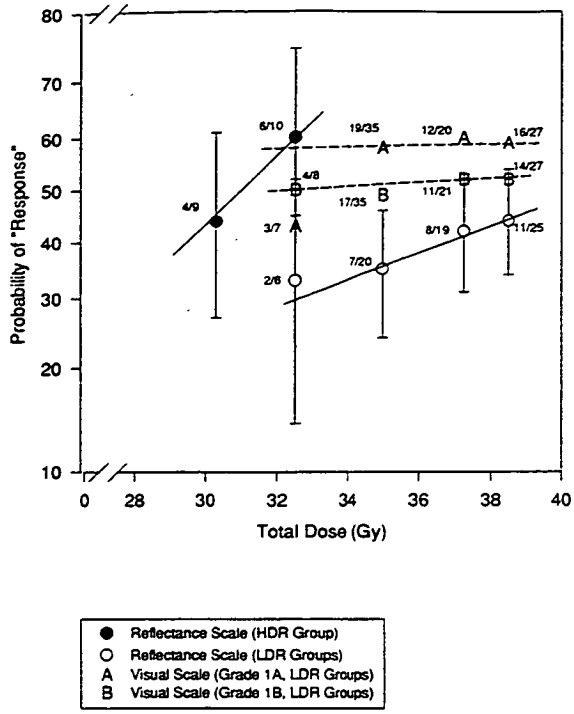

Fig. 5. Quantal dose-response plots (where response is defined as a peak erythema index $[E_p/E_0]$ exceeding 1.9, i.e., a percentage change greater than 90%) for patients treated at high dose rate and in all low dose rate groups combined. Closed squares represent response data for duration of reaction at Grades 1A and B for the patients treated at low dose rate, where response frequency corresponds to a peak erythema index of 1.9. (For 34 fields studied a full set of reflectance measures was not available.)

3.6. Factors influencing the dose response relationship

Generalised multiple linear regression models that examine the influence of two different sets of co-variates on peak erythema index are presented in Table 5. In both models increasing peak erythema index correlated positively and independently with total dose and dose rate predicting that doses of 30 Gy at high dose rate would produce identical erythema indices as doses in the range 36.5–37 Gy at 4.8 Gy/h. This corresponds well to the dose-response plots presented in Fig. 5. Increasing age, on the other hand, led to less marked rises in peak erythema index for the same given dose in both models. Although the binary variable sun exposure did not appear to exert an independent effect on peak erythema index, anatomical sites that are often the site of previous heavy sun exposure (namely the head and neck and upper torso) experienced lower peak erythema indices than less exposed sites.

In the first model presented in Table 5, females appeared to develop greater increases in erythema index than males, following the same dose, regardless of age, site or dose rate. In the second model, however, in which baseline erythema reading was included, the influence of gender remained but at considerably diminished strength. In this model baseline erythema emerged as a powerful inverse determinant of subsequent peak erythema index independent of the effect of total dose, dose rate, age, site or gender. In separate models (not shown) in which absolute $(E_p - E_0)$, rather than proportional (E_p/E_0), peak change in erythema was used as the dependent variable, the influence of gender retained significance in spite of the inclusion of baseline erythema in the models.

3.7. Factors influencing the rate of erythema development

Multiple linear regression modelling, summarised in Table 6, confirmed the impression suggested by the time

Table 5
Two generalised multiple linear regression models that examine the influence of a range of co-variates on the peak erythema measure obtained after radiation. Dependent variable = peak erythema index (E_p/E_0)

		Model 1			Model 2		
		Coeff	S.D.	p Value	Coeff	S.D.	p value
Total dose	(Gy)	0.06	0.02	<0.01	0.05	0.02	0.02
Dose rate	(Gy/h)	0.002	0.001	0.02	0.001	0.001	0.04
Head and neck	($y = 1$, $n = 0$)	−1.1	0.45	0.02	−0.66	0.42	0.12
Upper torso	($y = 1$, $n = 0$)	−1.0	0.46	0.03	−0.79	0.41	0.06
Lower torso	($y = 1$, $n = 0$)	−0.87	0.47	0.07	−0.81	0.42	0.06
Pelvic	($y = 1$,$n = 0$)	−0.94	0.47	0.05	−0.82	0.43	0.06
Arm	($y = 1$,$n = 0$)	−0.5	0.47	0.29	−0.4	0.43	0.35
Sun exposure	($y = 1$,$n = 0$)	−0.15	0.12	0.21	−0.09	0.11	0.39
Age	(years)	−0.01	0.004	0.01	−0.009	0.004	0.01
Gender	(m = 0, f = 1)	0.27	0.1	0.03	0.1	0.1	0.32
Baseline erythema	(units)	Omit	Omit	Omit	−0.03	0.01	<0.01
Intercept values		1.25	0.87	0.15	2.15	0.81	0.01

The models differ in that Model 1 does not incorporate the influence of baseline (pretreatment) erythema, which in Model 2 is seen to exert an independent influence. (Field size failed to exert an influence in models in which this co-variate was incorporated.) Omit, variable omitted from model; Coeff, regression coefficient; S.D., standard deviation of coefficient.

Table 6
Two generalised linear regression models that examine the influence of a range of co-variates on the rate of development of erythema following treatment. Dependent variable = rate of erythema development (erythema units/day)

		Model 1			Model 2		
		Coeff	S.D.	*p* Value	Coeff	S.D.	*p* value
Total dose	(Gy[a])	0.03	0.02	0.02	Omit	Omit	Omit
Dose rate	(Gy/h)	0.002	0.001	0.04	0.002	0.001	0.03
Head and neck	($y = 1, n = 0$)	−1.44	0.44	<0.01	−1.33	0.44	<0.01
Upper torso	($y = 1, n = 0$)	−1.76	0.43	<0.01	−1.67	0.43	<0.01
Lower torso	($y = 1, n = 0$)	−1.8	0.44	<0.01	−1.69	0.44	<0.01
Pelvic	($y = 1, n = 0$)	−1.87	0.45	<0.01	−1.77	0.45	<0.01
Arm	($y = 1, n = 0$)	−1.65	0.44	<0.01	−1.48	0.45	<0.01
Sun exposure	($y = 1, n = 0$)	−0.18	0.11	0.1	−0.19	0.11	0.1
Age	(years)	−0.008	0.004	0.04	−0.008	0.004	0.05
Gender	(m = 0, f = 1)	−0.19	0.1	0.07	−0.21	0.11	0.05
Baseline erythema	(units)	−0.01	0.01	0.28	−0.01	0.01	0.37
Dose intensity	(Gy/day[a])	Omit	Omit	Omit	0.24	0.16	0.14
Intercept value		2.12	0.85	0.01	2.32	0.68	<0.01

The models differ in that 'dose intensity' in Model 2 substitutes for 'total dose' in Model 1. (For the purpose of these analyses rate of erythema development has been assumed to be linear up to the time of peak erythema measurement and dose has been corrected for biological effect using $t_{1/2}$ values estimated by direct analysis for the complete data set, i.e., α/β 7 Gy and $t_{1/2}$ 0.06 h). Omit, variable not included in model; Coeff, regression coefficient; S.D., standard deviation of the coefficient.

course data (presented in Fig. 1) that dose rate exerts an influence on the rate of erythema development that is independent of other variables. Patients treated at high dose rate developed corresponding measures of erythema more rapidly than patients treated at 'low' dose rates. It is of interest that the influence of dose rate was independent of the influence of either total dose administered or dose intensity (in Gy/day or 'biologically equivalent' Gy/day) which exerted only modest (non-significant) influences themselves.

3.8. Indirect and direct quantal analysis

Because dose-response plots and multiple regression analyses (not shown) failed to demonstrate a dose rate effect in the range 3–8.2 Gy/h, data derived from all three groups treated in this range (i.e., 3, 4.8 and 8.2 Gy/h) were therefore pooled for the purpose of indirect analysis using the modified reciprocal dose methodology described in Patients and methods.

Fig. 6 presents a reciprocal dose plot fitted through isoeffect estimates derived from the multiple regression procedures. Because the parameter K depends on the repair constant μ, the slope of the reciprocal dose plot fits a range of permutations of α/β and $t_{1/2}$. The relationship, which is shown in the inset graph in the figure, is an inverse one. If a $t_{1/2}$ value of 0.04 h were considered appropriate, then the α/β ratio (intercept of the slope derived from the present data) would be 8 Gy. On the other hand, if $t_{1/2}$ values above 0.2 h were considered more appropriate, then the present data would be fitted by extremely low, or even negative, values of α/β. Direct analysis of the data using the $\alpha\beta$est program provided a measure of agreement with this relationship by converging on an α/β ratio of 6.9 Gy and a $t_{1/2}$ value of 0.06 h. It should be noted, however, that the program returned indefinite 95% confidence intervals for these estimates.

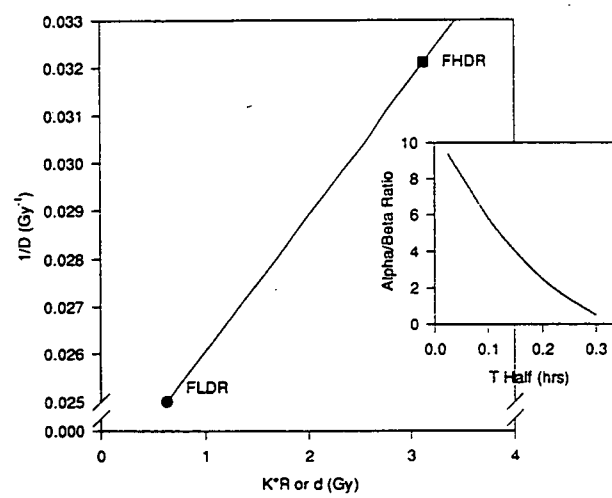

Fig. 6. A reciprocal dose plot based on the proportion of patients treated at high dose rate and the entire group of patients treated at different 'low' dose rates (i.e., 3–8.2 Gy/h) whose peak erythema index measure exceeded the median value for the whole study population. The parameter K depends upon the magnitude of the repair constant (see Dale et al. [8]). The inset graph therefore provides the relationship between the different permutations of α/β and $t_{1/2}$ that provide a fit to the data. The x axis of the main graph has been based on a $t_{1/2}$ value of 0.05 h and the data fit a slope that intercepts the x axis at −7.7 Gy. A fit to the data is therefore provided by an α/β ratio of 7.7 Gy and $t_{1/2}$ of 0.05 h. The inset graph suggests, however, that the fit provided by 4 Gy and 0.15 h, for example, might be equally as appropriate.

4. Discussion

4.1. Reflectance spectrophotometry

The colour of non-pigmented skin is largely determined by quantity of blood in the dermis, particularly in the sub-papillary venous plexus. Work by Dawson et al. [10] has confirmed the validity of simplifying the behaviour of human skin to that of a closed reflectance cell, where it is assumed that fibrous protein, collagen and fat do not contribute significantly to the reflectance spectrum and that the diffuse reflectance of the lowest collagen layer closely approximates one. Therefore, the pigments above this layer, (oxy-haemoglobin, deoxy-haemoglobin and melanin) are the chief light absorbing compounds which determine skin colour. The development of the science of photomedicine has stimulated the production of a number of reflectance instruments in the response to the need to precisely characterise the inflammatory response seen in a variety of dermatological conditions [1,6,18,20,31,32,39,40,51,52], since the visual assessment of erythema does not fulfil this criteria, even when aided by various comparative visual grading scales [3]. The reflectance spectrophotometer used in the present study has been shown to provide measures that are linearly proportional to haemoglobin concentration in both the in vivo and in vitro settings [10,19–21,23]. The unit utilises the entire optical wavelength for assessing the reflectance spectrum and incorporates algorithms to provide *independent* measures of haemoglobin index, its oxygen saturation and melanin content. Reflectance units used in other reported studies differ in that they sample at specific wavelengths and make no correction for melanin. This means that caution must be exercised when comparing the results obtained in the present project with previous studies performed elsewhere.

We have found the reflectance spectrophotometry unit employed in this study capable of reproducible erythema readings which typically varied by approximately ± 2–5 erythema units on otherwise unperturbed skin. There was no significant inter- or intra-observer variation related to the use of the unit and calibration tests utilising the Wratten filter produced similar constancy of measured output. The pilot study group results indicate a relative constancy of erythema in a comparable patient population measured under the same physiological conditions. Our control data demonstrated an intra-patient variability of 12% for this technique which compares with a value of 31% reported by Russell et al. [38]. The reflectance unit used was clearly more sensitive than the naked eye in detecting early radiation erythema. Our reflectance data also suggested the possibility of a decline in reflectance values during the second week of therapy that went undetected by the naked eye. This may correspond to the primary and secondary waves of erythema observed by early radiotherapists treating with orthovoltage apparatus. In some instances, the dip in erythema index fell below the baseline value suggesting the possibility that an in-field vaso-constrictive response may also be contributing the overall manifestation of the erythematous response. The relationship of erythema index to observed grade appeared to be relatively constant during the entirety of the reaction suggesting that other observable changes such as desquamation, oedema and increasing pigmentation, did not obviously influence the clinical scoring process with respect to erythema.

4.2. Comparison with qualitative data

A disappointing lack of concordance between measured erythema index and observed grade was evident with large intra- and even greater inter-observer variations leading to substantial overlaps in scoring Grade 1A, 1B and 2 skin reactions in this study. For the same reflectance erythema reading, greater reaction grades were assigned to females and at non-sun-exposed less pigmented treatment sites. To some extent, these variables are related, in that females generally tended to have less previous sun-exposure. It is possible to conclude however, that subjective grading in this study relatively under-estimated erythema in males and at heavily sun-exposed treatment sites. The reason for this observation is unclear. It is possible that the naked eye and the reflectance spectrophotometer respond to different signals. Perhaps subtle differences in the sub-epidermal vascular plexus of the type identified by Forbes and Argenbright [3] explain the discrepancies we observed between reflectance reading and erythema score in patients of different sex and at different sites. Alternatively, it may be easier to assign a reaction grade on a skin that is paler and less pigmented to start with. Daniel and Imbrey [9], discussed these problems in considerable detail and concluded that the human eye is an excellent device for sensing a quantal event (i.e., a change in appearance), but is inadequate in delineating graded difference of erythema or, most importantly, changes over time. In our study, the derived measures, erythema index (E_t/E_0) and change in erythema ($E_t - E_0$), reduced the influence of gender and site on reaction scoring. This is possibly because, as Daniel and Imbrey have pointed out, the eye is good at detecting changes which are effectively introduced by these derivations.

4.3. Dose-response relationships

While it is disappointing that we were unable to define a dose-response relationship using our qualitative scale of skin reaction, this can not be taken to mean that the scale based on the RTOG/EORTC grading criteria has no value in the clinic for comparing acute skin reactions.

It is pointed out that the range of doses used in the present study was relatively small and was in the palliative range. Very few patients experienced brisk erythematous reactions associated with significant desquamation. The present study therefore does not exclude the possibility that dose-response relationships might be derived in groups of patients treated to higher doses and who experience reactions than span the entire range of the scale used (i.e., including desquamation). For example, Dutriex and colleagues [15] utilised the ability to discriminate between different grades of desquamation to produce a dose-response curve for human skin with relatively small patient numbers by simultaneously comparing supra-clavicular reactions in the one patient. Data from the present study merely show that reflectance spectrophotometry is a more reliable instrument for measuring erythematous skin reactions than the naked eye.

Although more reliable than the naked eye, reflectance measures did not provide the well-defined relationships between dose administered and erythematous response that we were hoping for. Interpatient variability in reflectance response and choice of the appropriate measure of erythematous change proved to be the greatest problems. The failure to derive meaningful dose-response relationships (i.e., with positive slopes and reasonable correlation coefficients) using our raw peak erythema (E_p) data quite probably stems from the fact that peak erythema is so strongly dependent on initial erythema measure and that this measure varied considerably in all patient subsets. The use of a control erythema obtained simultaneously in unirradiated skin outside of the field to correct for systematic variations and transient changes in erythema is a theoretically appealing method of assessing change in erythematous measure due to treatment. In practice, however, selection of an appropriate control site can be difficult. In the present study, for example, in which we used a point 3 cm from the field edge, we observed clear rises in erythema outside of the field that paralleled rises within the field in approximately a quarter of all patients. This phenomenon, which was recognised by Jolles and Mitchell in 1947 [29] in patients treated with orthovoltage apparatus (and called by them 'the reciprocal vicinity effect'), suggests the possibility that humoral factors produced within the field alter capillary blood flow both inside and outside the field for some distance. Although no patients enrolled in the study had overt skin involvement by tumour, underlying nodal or bony disease may also have had local effects on blood flow influencing erythema values obtained. This hypothesis is supported by the fact that pre-treatment reflectance measures exceeded control measures by up to 3 units in approximately 20% of patients.

Peak measures of *proportional* change in erythema (peak erythema) index (E_p/E_0) and percentage erythema increase ($[E_p - E_0]/E_0 \times 100$) probably provided better dose-response relationships in this study, in all patient subsets, because these manoeuvres reduce the effect of variations in the initial erythema. Baseline erythema distributions are negatively skewed in all patient subsets and division of subsequent readings by baseline erythema tends to normalise and reduce the variance of the distribution of these values. Duration of erythema at given levels above baseline and area under the reaction curve probably did not provide better dose-response relationships than peak erythema index because these parameters were so highly correlated. In a separate report [12] we have pointed out that *duration* of oropharyngeal mucosal reactions at a given grade can provide a more precise measure of effect than peak grade itself (an ordinal measure). This is because a range of 'biological' doses will produce the same ultimate reaction grade. However, erythema index measured by reflectance spectrophotometry is a continuous variable, and is shown herein to be a more sensitive measure of erythema than erythematous grade assessed by the naked eye.

4.4. Radiobiological implications

In our multiple regression analyses, the range of factors found to influence baseline erythema value, rate of erythema development and peak erythema measure independently of the dose actually delivered is interesting because their influences are not well documented in the literature. The finding that baseline reflectance erythema values are higher in males and at sun-exposed sites such as the head and neck is not surprising, however, because it is to be expected that patients with higher serum haemoglobin concentrations (e.g., male patients) and sites where chronic vasodilatory changes are present (e.g., heavily solar damaged sites) should have greater haemoglobin concentrations measured in the subpapillary plexus.

Although the multiple regression models that addressed the influence of factors other than dose on change in erythema due to treatment suggested some causes for inter-patient variation in response, it must be cautioned that the models did not explain the entire variation witnessed in the study. This is not particularly surprising in itself. The study by Russell et al. in Amsterdam [38], which focussed on one treatment site in one gender, identified significant variations in inter-patient response to similar radiation doses. Gender, advancing age and site emerged in the present study as the most powerful sources of inter-patient variation in response. However, how these factors can influence the haemoglobin content of the subpapillary plexus after irradiation is more difficult to explain. Unless basal cell radiosensitivity varies with these factors, it must be postulated that factors other than basal cell killing influence degree of vasodilation that occurs in response to radiation. Unfor-

tunately little is known about the mediators of radiation erythema. Inflammatory mediators such as the ecosanoids and related compounds, TGF, TNF and other cytokines [2,5,27,28,30–32,36,37] have been implicated in the expression of erythema and it is thought that the pre-capillary sphincter represents the major vascular target for mediators such as these. Other mediators such as histamine and ϵ-amino caproic acid may also be involved in the production of erythema via altered vascular permeability [16,37]. From work performed in relation to ultraviolet-induced erythema [30] there is considerable evidence to indicate that a vasoactive mediator released from the epidermis, diffusing into and around dermal blood vessels, represents the most appropriate model for the generation of UV-induced erythema. Our data suggest that the reactivity of the subpapillary plexus is at least in part influenced by gender, increasing age and, probably, by prior solar damage. However, whether these factors influence the release of vasoactive substances or alter the reactivity of the target sites for these substances can not be established from the present study and will remain a matter for speculation until studies addressing these phenomena are undertaken.

The failure of this study to demonstrate dose rate effects in the range 3–8.2 Gy/h (Fig. 4) does not necessarily mean that dose rate effects in this range do not exist. Substantial inter-patient variations associated with heterogeneities in the dose rate subsets with respect to age, gender site, etc. (see Table 2), could have quite easily obscured differences due to dose rate in reflectance response over the limited dose range studied. The differences in dose response demonstrated in the regression models shown are therefore almost entirely due to differences in erythema produced in the *entire* group of patients treated at 'low' dose rate and the group treated at high dose rate. Whether these differences are exclusively due to differences in basal cell killing in the two dose rate ranges is a moot point, however. The finding that rate of erythema development inside the field was more rapid in patients treated at high dose rate independently of the dose administered or its intensity, together with the finding that patients treated at high dose rate also had more intense out of field erythema changes, could suggest the presence of a separate mechanism of erythema induction that is not related to basal cell killing, but which is also dose rate dependent.

The estimates for the α/β ratio and repair half times derived by indirect and direct means in this project have more interest value than true meaning in our opinion. There is little doubt that the size of inter-patient variations in reflectance response, together with heterogeneities in the data set and the limited range of doses studied, contributed to the indefinite confidence intervals around the direct estimates of 6.9 Gy for α/β and 0.06 h for repair half time obtained. However, the value for α/β estimated from the present data set is not dissimilar from previous estimates for treatment courses of 2–3 weeks duration [43,49]. It is the repair half time estimate that is remarkably short and which deserves further consideration [41]. The indirect analysis depicted in Fig. 6 suggests that more plausible values for $t_{1/2}$ would not be compatible with the data unless much lower (and even negative) values of α/β are countenanced. Of course, the incomplete repair model used in our analyses assumes monoexponential repair kinetics. If this assumption is incorrect for the basal cells of the human skin, as already suggested by Turesson and Thames [49], and if an extremely rapid repair process that is completely overwhelmed by relatively small fractional doses delivered at high dose rate were to exist, then the incomplete repair model could return very rapid half time values from data derived from studies comparing treatments administered at high and low dose rates (such as the present one). However, to return a value as low as 0.06 h, the proportion of the overall repair process contributed to by a rapid process, that has a half time shorter than 5 min, would need to be substantial. For this reason, we suspect that dose rate-dependent mechanisms of erythema production unrelated to basal cell killing could be responsible for the apparent paradox provided by our analyses.

Although erythema has long been held to be dependent on basal cell killing, it has often been speculated that the relationship is not straightforward and that other factors may be operative. Recently Nyman and Turesson have shown that reflectance measures of erythema bear a relationship with basal cell density measures in women treated with a variety of fractionation schemes in the management of primary breast cancer [35]. However, paradoxical results in a group of these patients treated on an accelerated fractionation regimen suggested that erythema measure and basal cell density may not reflect total basal cell kill. The present study has been helpful because it has identified factors other than basal cell killing that may influence the erythematous response and which may be amenable to further study.

We are currently completing a reflectance project using varying fraction numbers at high dose rate which we hope will provide further data that will help to better define the relationship between erythema induction and increasing dose at different dose rates. In future studies we will heed an important lesson from the present study by ensuring that treatment allocation will occur following stratification according to gender, age and site, or be confined to one site in one gender.

5. Conclusions

We conclude that:

- The erythematous component of the acute skin reac-

tion in humans is assessed more reliably using the reflectance spectrophotometer than by eye. The unit used by us was sensitive to out of field effects and dips in erythema during the first and second weeks of therapy that went undetected by eye. It was also sensitive to differences in baseline erythema according to treatment site, gender and previous UV exposure and enabled the generation of derived measures that correlated with increasing dose delivered more satisfactorily than increasing reaction grade scored by eye.

- Factors unrelated to dose such as age, gender as well as prior sun exposure also contribute to the degree of erythema that occurs following irradiation. These factors have contributed to but do not entirely explain the substantial inter-patient variation in response observed in this study.
- Dose rate is a factor that influences both the degree of erythematous change and the rate at which the change occurs. Mechanisms other than basal cell killing, however, are implicated by the data.
- The α/β ratio and $t_{1/2}$ values estimated from the present data require cautious interpretation due to the operation of mechanisms of erythema induction and/or its modification other than basal cell killing.
- Further work to define the pathophysiological mechanisms of erythema induction are necessary.
- In future radiobiological studies treatment allocation should follow stratification according to gender, age, site and prior solar damage, or be confined to one site in one gender.

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UNDERPREDICTION OF HUMAN SKIN ERYTHEMA AT LOW DOSES PER FRACTION BY THE LINEAR QUADRATIC MODEL

Christopher S Hamilton *
James W Denham *
Maree O'Brien *
Patricia Ostwald *
Tomas Kron *
Suzanne Wright *
Wolfgang Dörr ‡

* Radiation Oncology Department
Newcastle Mater Misericordiae Hospital
Hunter Region Mail Centre NSW 2310 Australia

‡ Klinik und Poliklinik für Strahlenbiologie
UK Carl Gustav Carus
Dresden Germany

Requests for reprints -

Dr C S Hamilton
Radiation Oncology Department
Newcastle Mater Misericordiae Hospital
Locked Bag 7
Hunter Region Mail Centre NSW 2310 Australia
Telephone +61 49 21 1177
Facsimile +61 49 60 2566

Running Head: **Underprediction of human skin erythema**

ABSTRACT

The time course of erythematous skin reactions has been recorded using a reflectance spectrophotometer in 65 patients treated palliatively with 5, 10, 12 and 20 daily treatment fractions using different thicknesses of bolus in each treatment field to vary dose per fraction across the field at the skin surface. Skin doses in these patients ranged between 0.4 and 5.2 Gy/fraction. Reactions have also been recorded in the pubic region of an additional 52 patients undergoing prostatic irradiation using 30 -32 fractions. Doses at the skin surface in these patients was estimated to be approximately 0.4 Gy per fraction.

The data provide support for findings from a previous study that gender, age, site and prior sun exposure influence pre-treatment erythema values and that these factors also influence post-treatment erythema values independently of the radiation dose administered. In addition, the finding that faint erythematous reactions at least 3 cm outside of the irradiated field can be recorded is also confirmed.

Unexpectedly intense erythematous reactions were observed at doses per fraction below 1.5 Gy, at the skin surface. This suggests either that the dose response relationship for erythematous skin reactions is not linear quadratic, *or* that erythema is not exclusively initiated by damage to the basal cell layer as widely held. An "s" shaped curve appears to fit the dose response data more satisfactorily. This finding warrants confirmation and exploration in further purpose designed studies.

Keywords: skin, erythema, radiotherapy, reflectance spectrophotometry, α/β ratio, fractionation parameters

INTRODUCTION

Radiotherapists have been observing the effects of ionising radiation on mammalian skin for the better part of the twentieth century. Quantification and qualification of acute radiation effects in mammalian skin systems, such as the mouse, rat and pig, have received extensive attention in the radiobiological literature, however, apart from the work of Turesson, relatively little systematic scientific study of the radiation response of human skin has occurred. [1,5,9,11,12,15] With increasing application of the linear quadratic model to experimental data since 1982, derivation of α/β ratios and t½ values for acute effects in human skin over a variety of anatomical sites would clearly be useful to augment animal data. Fractionation effects and repair values are required from clinical radiobiological studies for the safe and rational design of new radiotherapeutic protocols (eg hyperfractionated or accelerated radiotherapy). Although the skin may not always represent the critical dose-limiting normal tissue for megavoltage radiotherapy, knowledge of its response is important for modelling of other acute reacting tissues and for treatments with electron or proton beams. The initial stimulus for this project arose from the observation that there existed no systematic quantitative observations on the effects of fractionated low dose rate (FLDR) radiotherapy in human skin. We have previously reported these results [4] and demonstrated that a variety of physiological factors impact on the local erythema response over and above the physical dose delivered. These include gender, site of irradiation and age. This report also included 19 patients treated with 10 fractions at high dose rate. In order to expand this work to cover a larger range of fraction numbers and doses per fraction, further studies were designed. This report describes the results obtained with reflectance spectrophotometry in two prospective fractionated high dose rate clinical radiobiological studies.

PATIENTS AND METHODS

1 Patients

1.1 *Palliative reflectance study*

This group consisted of patients with incurable and almost invariably metastatic malignancy, having symptoms justifying palliative radiation treatment. Consenting patients were of the following fractionation schemes:

20 Gy/5 fx (n=12)

30 Gy/10 fx (n=25)

36 Gy/12 fx (n=14)

40 Gy/ 20 fx (n=14)

Exclusion criteria included previous irradiation to the area concerned, previous history of significant dermatological illnesses, current or recent known photosensitising drug intake, and poor performance status (ECOG > 2). Written informed consent was a prerequisite for participation in this study, which was approved for activation by the Hunter Area Regional Ethics Committee in March 1991. A breakdown of the treated group according to age, gender, treatment site and prior heavy sun exposure is presented in Table 1.

1.2 *Hair cortical cell counting project (HCCC)*

Patients undergoing conventional external beam radiotherapy (60-65 Gy using 30-32 daily fractions) for localised carcinoma of the prostate were eligible for this study whose intention was to evaluate the response of hair follicle cortical cells to X-rays as well as erythema (at the supra-pubic site only). Written informed consent was a prerequisite for participation in this study, which was approved for activation by the Hunter Area Regional Ethics committee in April 1993.

2 Irradiation techniques

2.1 *Palliative reflectance study*

Patients treated on the "palliative reflectance study" were irradiated with 6 MV photons at 100 cm SSD on a Varian Clinic 1800. Sites of treatment were confined to the truncal portion of the body between larynx and groin. Field configurations included single direct fields, or parallel opposed pairs. In order to generate a greater number of dose points, patients with field sizes between 100 cm^2 and 175 cm^2 , had their field arbitrarily divided up into two sections. The first of these sections was treated through a layer of full wax bolus (for 6 MV photons, 1.5 cm). The second of the sub-divisions of the treatment field was treated with no bolus. This resulted in approximately a 30 to 40% dose differential to skin in the two areas concerned. For field sizes greater than 175 cm^2, the treated field was artificially divided up into three sections in an analogous manner with, on this occasion, the third section being treated daily through a layer of thin wax bolus (0.75 cm). Fields smaller than 100 cm^2 were treated without bolus. A breakdown is provided in Table 1. Reflectance readings were obtained in the centre of each of these arbitrary sub-divisions of the field and at 3 cm outside of the field, as shown in Figure 1.

2.2 *Hair cortical cell counting project*

Patients were treated using 6 MV photon with a four-field "box set-up" (AP/PA plus opposing laterals) to a target absorbed dose of 60-65 Gy in 30-32 fractions. No bolus was used. Reflectance readings were performed at the centre of the anterior field only, i.e. in the suprapubic region. Care was taken to separate the test and control points from the hair sampling point. Fields were CT-planned to cover the prostate gland plus a 1.5 cm margin only. Full details of the hair

cortical cell counting technique are given in Potten (1994) [10]. The technique involved removal of approximately 100 groin hairs prior to treatment and again after a given dose (in this case 4, 8, 16 or 32 fractions). Hair cortical cell density has been measured using confocal microscopy and the fractional reduction has been used as a measure of follicle

cell kill.

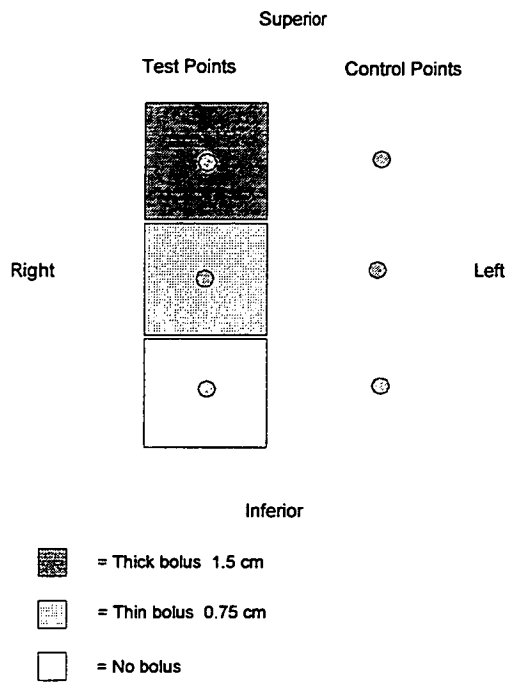

Figure 1

Schematic representation of the field sub-division (total field area greater than 175 cm^2) into differentially bolused areas along with test and control points for reflectance readings in the "palliative reflectance" study.

3 In vivo dosimetry

Paired carbon-loaded TLDs (Vinten) [13] were inserted into a tissue equivalent polystyrene holder and applied to the field centre on the patient's skin. No additional build up was utilised for these measurements. Where 0.75 cm and 1.5 cm of bolus was present on portions of the field, a pair of conventional lithium fluoride (LiF) TLDs were placed beneath the bolus. In all dose-response analyses subsequently described on this report, measured skin dose rather than prescribed dose has been used. A breakdown of measured doses appears in Table 1.

4 Reflectance technique

The reflectance unit employed in this study (Spectral Research Pty Ltd) was designed specifically for quantitative haemoglobin estimation in human skin [6] and its evaluation in the context of human radiation induced erythema is described by Denham et al (1995) [4]. Reflectance readings were obtained prior to treatment and at weekly intervals during and after the course of radiotherapy, until visible evidence of the reaction had subsided. Measurements were obtained with the patient in a recumbent position, in an air-conditioned environment, at a constant ambient temperature of 22°C after a five minutes rest period. Selected measurement sites were referenced using a small non-permanent skin mark which were consistently aligned on subsequent measurements with a reference mark on the external surface of the measurement probe. The mean of five separate measurements was taken to represent the reflectance reading for that day. "Control" measurements were obtained 3 cm outside of the field (Figure 1).

In this report change in erythema as a result of radiation is expressed as "percentage change". This quantity is

$$\frac{E_t - E_o}{E_o} x100$$

Where E_o is the erythema measure immediately prior to treatment (i.e. the baseline measure) and E_t is the measure at any time t subsequently (i.e. during or after treatment).

5 Analytical techniques

All data has been stored on a temporal database (MEDLOG) which permits a limited range of statistical manoeuvres, including multiple linear regression, to be performed. Indirect analysis was based on isoeffect estimates by probit analysis of peak "percentage change" data. In these analyses and in direct analyses using αβest [2] a "response" was assigned if a patient's peak percentage change value equalled or exceeded the median peak value recorded for all patients treated in both studies.

RESULTS

1 Factors that influence pre- and post-treatment erythema values

Multiple linear regression procedures in which anatomical site was arbitrarily subdivided into four categories (anterior and posterior-chest regions down to the bottom of the rib cage, anterior abdomen to include the pubic region and groins, and finally lumbar-sacral to include lumbar regions and buttocks) suggested that heavily sun exposed areas such as the upper torso had significantly greater pre-treatment erythema values than other sites independently of other factors such as age and gender ($p<0.05$). Non significant trends towards greater pre-treatment erythema values ($p=0.05$-0.2) were noted in patients of increasing age. Similar models that addressed the influence of factors other than total radiation dose delivered and dose per fraction, such as age, gender, site, sun exposure and pre-treatment erythema value on the peak percentage change in erythema recorded during or after radiation were also developed. These suggested that lower pre-treatment erythema values are significantly associated with greater peak changes in erythema independently of other factors including total dose ($p<0.01$). Non significant trends towards younger patients developing greater peak changes in erythema ($p=0.05$-0.2) were also noted.

Addition of these data sets to the "low dose rate" data set analysed in a previous report in this journal [4] amplified the statistical significance of findings presented in that report. To summarise the results for the combined series of 183 fields with complete data higher pre-treatment erythema are associated with male gender ($p<0.01$), older patients ($p<0.05$), and at sun exposed anatomical sites ($p<0.01$). Greater post-treatment erythema, on the other hand, are associated with high instantaneous dose rate ($p<0.01$), lower pre-

treatment values ($p<0.01$) and younger patients ($p<0.05$) independently of total dose administered or dose intensity. A non significant trend towards female patients developing greater changes in erythema following treatment ($p=0.1$) was also present.

2 Erythema according to quantity of bolus used on the skin surface

In Figure 2 peak erythema according to total dose has been presented for the four fractionation groups in which different quantities of bolus were applied to the skin surface during treatment in the "palliative reflectance" study (i.e. 5, 10, 12 and 20 fraction groups). Best fit non-linear regression lines have been fitted through data points obtained from areas of the field that were unbolused, (i.e. received doses between 16 and 47% of D_{max} [median 33.7%] at the skin surface), areas bolused with 0.75 cm of tissue equivalent material (i.e. received between 88 and 100% of D_{max} [median 94%] at the skin surface) and areas fully bolused with 1.5 cm of tissue equivalent matter (i.e. that received 100% of the estimated D_{max} dose). Although higher peak percentage change values have been recorded in the fully bolused areas in each fractionation group it will be noted that the dose response relationship for measured values across all areas of the field is not linear or linear quadratic, with higher peak percentage change values recorded in unbolused areas in all four fractionation groups than predicted by the linear quadratic model using any positive value for α/β.

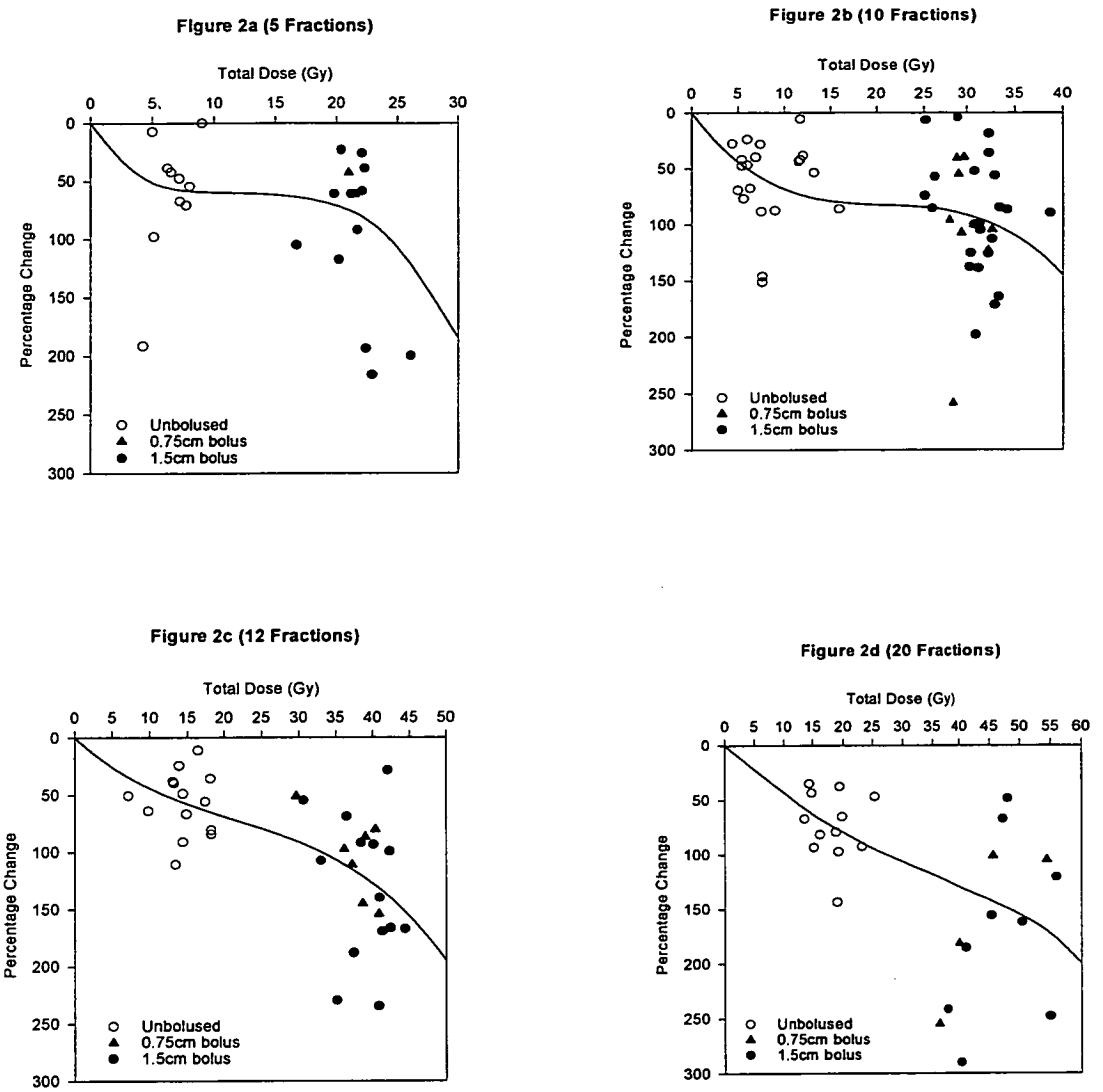

Figure 2

Peak percentage change in erythema recorded in the four fractionation groups of the "palliative reflectance" study according to the total measured dose at the skin surface.

3 The time course of reactions inside and outside of the field

The maximum erythema values recorded at each attendance in each field have been used to compose the time course data for each fractionation group presented in Figure 3. Rate of development of erythema is similar for the 10, 12 and 20 fraction groups (i.e. dose per fraction in the range 2-3.4 Gy/fraction or 10-17 Gy/week) but appears more rapid in the 5 fraction group (approximately 4.4 Gy/fraction or approximately 22 Gy/week) and more slowly in the 32 fraction group (approximately 0.4 Gy/fraction or 2 Gy/week). Of note is the observation that the rate of development of erythema and peak percentage change reached in the 32 fraction (0.4 Gy/fraction or 2 Gy/week) group is so great, and considerably greater than predicted by the linear quadratic formula using any positive value for α/β.

Figure 3

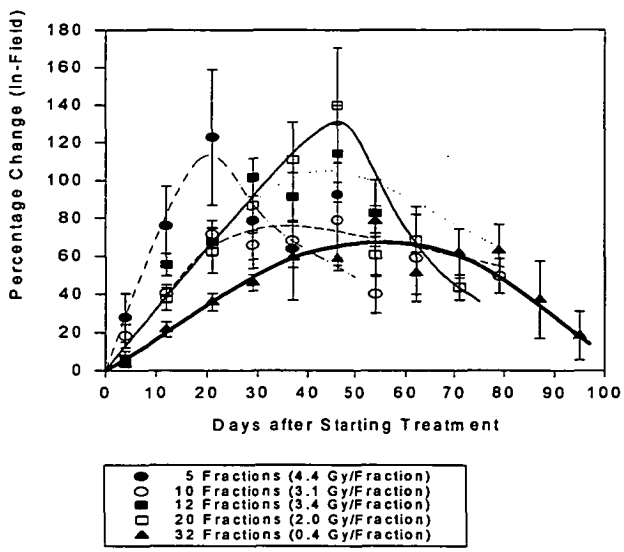

Figure 3

The time course of reactions inside of the treatment field grouped according to the number of fractions delivered.

In 39 (60%)of the 65 patients treated in the "palliative reflectance" project (the 5, 10, 12 and 20 fraction groups) increases in erythema outside of the treated field, were noted. These changes have been grouped and plotted in Figure 4. From this figure it would appear that the greatest out of field erythematous changes occurred in the highest dose per fraction group (i.e. the 5 fraction group that received dose per fraction of approximately 4.4 Gy/day).

Figure 4

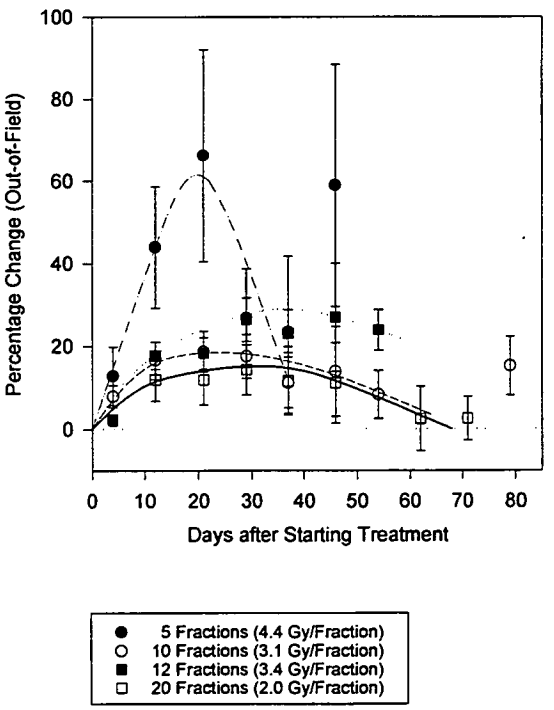

Figure 4

The time course of "reactions" 3 centimetres outside the field in a "control" area grouped according to the number of fractions delivered.

4 Indirect and Direct analyses

Probit analyses have been used to derive "isoeffective doses" for the various fractionation groups. For the purposes of these analyses the median peak percentage change value for the entire data set (75%) has been used to derive the cut point between a "responding" patient (whose peak percentage change equals or exceeds 75%) and a "non-responding" patient (whose peak percentage change never reaches 75%). In Figure 5 a reciprocal dose (Fe) plot is presented using these estimated isoeffect dose at the 0.5 probability of "response" level for each fractionation group. It will be noted that the data points do not lie on a single straight line. In particular, the data points for the 20 fraction and the 32 fraction group are higher against the reciprocal total dose axis (i.e. the "isoeffective dose" is lower) than expected. In the figure two regression lines have arbitrarily been fitted. The first, which fits the 5, 10 and 12 fraction data points, extrapolates back through the dose per fraction axis at -8.5 Gy (to provide an α/β ratio of 8.5 Gy). The second which passes through the data points for the 20 and 32 fraction groups extrapolates through the dose per fraction axis at approximately 1.6 Gy (to provide and α/β ratio of -1.6 Gy!). As expected the data also failed the de Boer test [3] for a linear quadratic fit to the data (Figure 6). This confusing picture is reflected in the results produced by direct analysis using the $\alpha\beta$est program. An α/β ratio of -5 Gy (indefinite confidence intervals) is returned for the "palliative reflectance" project data alone (i.e. 5, 10, 12 and 20 fraction groups). When both data sets are analysed together, $\alpha\beta$est converges on an α/β ratio of -9 Gy (again with indefinite confidence intervals). These negative values are due to negative values for β.

Figure 5

Indirect Analysis

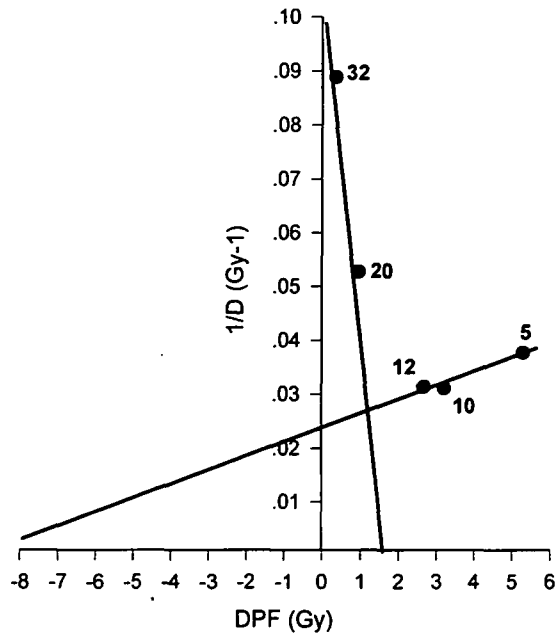

Figure 5

Reciprocal dose (Fe) plots based on isoeffective doses for the five fractionation groups calculated from probit analysis. Two regression lines have been arbitrarily fitted through the data points. One which fits through the 5, 10 and 12 fraction groups extrapolates to an α/β of 8.5 Gy. The other which fits the 20 and 32 fraction groups extrapolates to -1.6 Gy.

Figure 6
de Boer Plot: (FHDR)

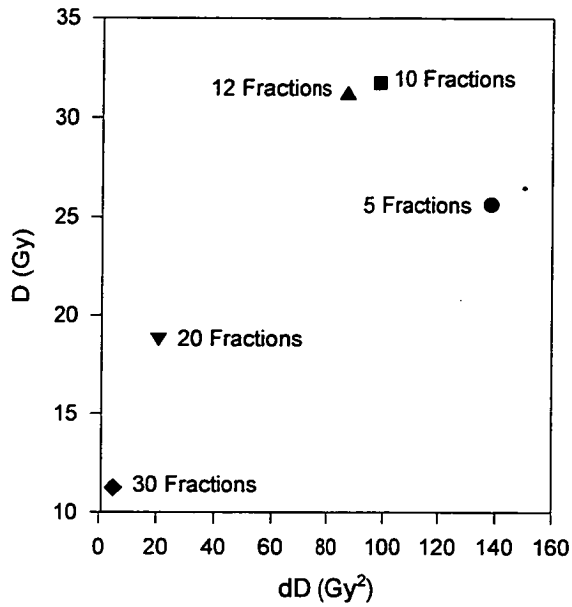

Figure 6

De Boer plot [3] for isoeffective data from the two studies. The linear quadratic model would be satisfied if the data points were to lie on a straight line.

DISCUSSION

When this series of studies commenced we were unaware of how factors such as gender, age, site and prior sun exposure can influence erythematous reactions **independently** of the effects of radiation dose. It was only after analysis of reflectance data from our "Low Dose Rate" project that we came to appreciate the importance of these factors [4]. By then, however, it was too late to prospectively stratify accrual according to these factors as subsequently recommended by us. As a result therefore, the precision of the results obtained in the present studies is not as great as it could have been. This disappointment, however, was partly offset by confirmatory support from the new data sets that factors such as pre-treatment erythema level, age, site and possibly gender can exert an influence on erythematous skin reactions that is independent of dose.

Fortunately, the imprecision of our data was not too great to obscure another potentially important finding. This was that low doses per fraction (i.e. under 2 Gy) produced far more intense erythema (as measured by reflectance spectrophotometry) than the linear quadratic model would have lead us to expect. We were disinclined, however, to believe the first indication from our data that this was true. This indication came from fields treated in the "palliative reflectance" study (5, 10, 12 and 20 fraction groups) where portions of each field had been left unbolused and portions had been bolused with 0.75 cm and 1.5 cm of tissue equivalent material (Figure 2). The peak erythema measured in the unbolused areas were greater than anticipated. It was believed that this finding could be due to the release of vasoactive mediators or the stimulation of neurovascular reflexes, from the adjacent area of the field that had been bolused during treatment (i.e. from the area in the field receiving the highest dose at the basal cell layer). This was thought to be plausible because unexpected erythema during the time course of the in-field reactions had been measured at least 3 cm **outside** of the irradiated field altogether in a

significant proportion of cases (Figure 4). What made us more confident that the unexpectedly intense erythema at low doses per fraction was a real finding, however, were the peak erythema recorded at doses per fraction to the order of 0.4 Gy in the "hair cortical cell counting" project (32 fraction group) (Figure 3). Although these data came from non exposed sites in male patients their credibility was less open to doubt due to the quantity of observations obtained and from the fact that they were made of an identical site in patients of a similar age.

Several possibilities were considered to explain the unexpectedly intense erythema observed in the unbolused areas in both studies. With the 6 MV beam used dose builds up rapidly below the skin surface and approaches 90% of D_{max} occur at approximately 7-9 mm below skin surface. If, in addition to mechanisms associated with basal cell killing, neurovascular reflex vasodilatation in response to so far undefined dermal/subdermal radiation effects is a contributor to erythema, then a potential explanation for unexpected erythema in unbolused areas is to hand. Unfortunately, however, we cannot offer, nor know of, any data to support this contention. It was also thought possible that the lack of prior sun exposure and the pre-treatment pallor of the skin in unbolused areas, were contributing factors to the unexpectedly intense erythema measured. Although our multiple regression models confirmed that this was a possibility for the measurements obtained in the pubic area, however, they failed to predict the unanticipated erythemas measured in the unbolused portions of the fields treated in 5, 10, 12 and 20 fraction groups. A further possibility considered was that our reflectance spectrophotometer provides non-linear readouts in erythematous response. We wondered if our unit could exaggerate the magnitude of faint erythema but not intense erythema. While it is true that an informal comparison of our unit with the units at Gothenburg and Amsterdam using various test materials confirmed differences in the responsiveness of the three units, there was no suggestion that our unit would provide readouts any less linear than the other two (with thanks to Dr Nicola Russell,

Amsterdam and Dr Ingela Turesson, Gothenburg, August 1995). In addition, tests performed in the development stage of our unit were also considered to confirm linearity in the unit's response [6]. Another explanation considered for the intensity of the erythema in the "hair cortical cell" project (the 32 fraction group), in particular, was the possibility that recruitment of cells into the cell cycle as part of a proliferative response to basal cell damage, which is believed to start between 16 and 28 days after the start of radiation treatment [7,15], could lead to sensitisation of the cellular population with subsequent release of vasoactive factors out of proportion to the size of continuing radiation doses delivered after this point in time. Making this explanation less plausible, however, is the information presented in Figure 3. In this figure it is noted that disproportionately great increases in erythema for the 0.4 Gy/fraction patients are noted long before day 16 of treatment.

A further possibility is that the radiation dose response curve for erythema is, in fact, "s" shaped as suggested in Figure 7. However, caution needs to be exercised in interpreting this pair of figures. In Figure 7a, where the peak erythema which each patient has experienced has been divided by the total number of fractions delivered, the fact that the higher dose per fraction treatments were given over a shorter time frame than the lower dose per fraction treatment (i.e. the time factor) is ignored completely. It was felt that the presentation in Figure 7b circumvents this problem by presenting the rate of development of the erythematous reaction in each patient during the development of the reaction (i.e. the mean change in absolute erythema measure per day).

Figure 7a

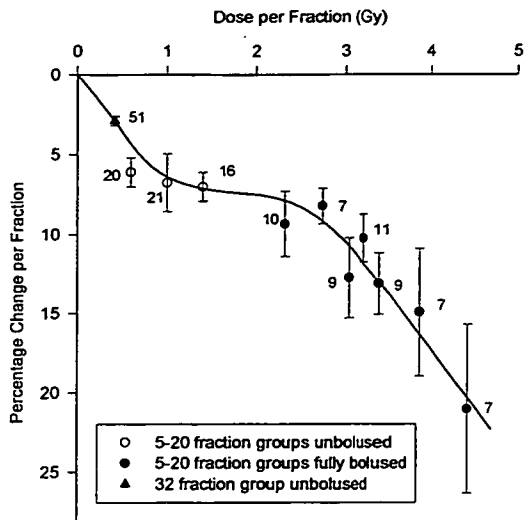

Figure 7b

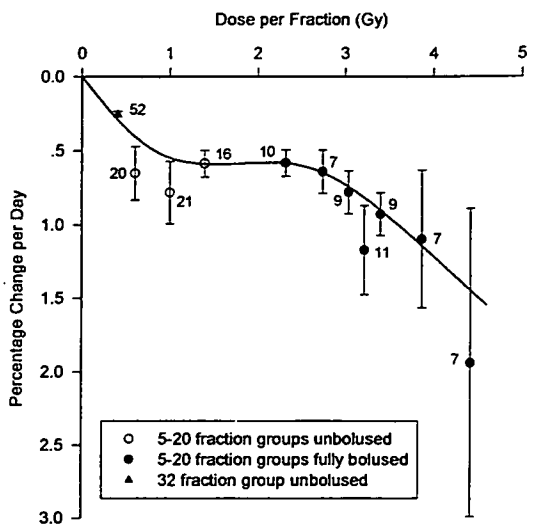

Figure 7a

Percentage change per fractional dose (i.e. peak percentage change/number of fractions) according to dose per fraction for patients treated in both studies.

Figure 7b

Percentage change in erythema per day during the evolution of reactions according to dose per fraction.

At first sight it would be tempting to speculate that the "s" shape of the dose response curve presented in these figures is caused by a failure to induce the reparative enzymes responsible for some of the simpler DNA lesions that cause "sublethal" radiation damage, at low doses per fraction. This explanation has been proffered by Joiner et al to explain greater than expected effects at low dose per fraction in several laboratory experiments [8]. However, this explanation may not be appropriate because these presentations make an assumption which could be incorrect, namely, that each new erythematous stimulus adds (quantitatively) to the previous one. If this assumption is incorrect and erythematous stimuli caused by low doses per fraction **were** to add to greater degree than erythematous stimuli due to higher doses per fraction (perhaps due to the temporary saturation of receptor sites for mediative vasoactive substances or for the mediative processes in the subpapillary micro-vasculature themselves) then a "s" shaped dose response curve could be produced by this type of analytical manoeuvre. Another point of potential importance is the fact that the effect noted by Joiner et al has been observed at doses of less than 0.6 Gy/fraction.

Whatever explanation is preferred for the apparently "s" shape of the dose response curves, presented in Figure 7, there is quite clearly a need to confirm this unexpected suggestion by conducting a further **purpose designed** prospective study. If the finding is confirmed it will then be necessary to find an explanation for it that is compatible with the dose modifying effect demonstrated by Turesson et al when intervals of 15 minutes are introduced in the same fraction of treatment [14], and by us when fractional doses are prolonged to more than 30 minutes in duration by their delivery at intermediate dose rates [4]. In Turesson's study a gap of 15 minutes during the delivery of a 7 Gy fraction was sufficient to reduce its "biological effectiveness" as measured by reflectance spectrophotometry, by approximately

by an approximately similar amount, when delivery was prolonged over 30 minutes. Both of these observations would tend to suggest either that a rapid reparative process is operative or that refractoriness in the erythematous mediative pathways is induced quite rapidly.

Perhaps it need not be pointed out that a "s" shaped dose response relationship for erythema provides a satisfactory explanation for the variety of α/β ratios that were obtained by indirect and direct analyses. If the erythematous dose response curve were truly "s" shaped it would be expected that different values for the α/β ratio would be obtained depending on which portion of the dose response curve data used in the analysis came from. For example, if data obtained at doses per fraction greater than 1.5 Gy are isolated then a negative α/β ratio of approximately -5 Gy is obtained by direct analysis. On the other hand, data below 1.5 Gy per fraction are isolated then a negative α/β ratio of -9 Gy is obtained. A positive α/β ratio of 8-9 Gy can be obtained by indirect analysis of data above 3 Gy per fraction! (Figure 4). This possibility could provide an additional explanation for the changes in α/β ratio noted by Turesson and Thames [15] and Hopewell and Aardweg [7] in studies where different experimental designs have been used.

CONCLUSIONS

- The finding from our previous study that pre- and post-treatment reflectance values vary according to gender, age, site and prior sun exposure is confirmed.

- Unexpectedly intense erythema following low dose per fraction treatments suggest either that the dose response relationship for human erythema is not linear quadratic, or that erythema is **not** exclusively initiated by damage to the basal cell layer. Prospective purpose designed studies are necessary to confirm this finding, however.

ACKNOWLEDGEMENTS

We should like to thank the many workers around the world who have helped us with the analysis and interpretation of the data.

We are also grateful to the radiography and physics staff of the Mater Hospital Radiation Oncology Department for their support of the projects, and to Ms Jennifer Hutchings and Mrs Allison Steigler for preparing the manuscript.

	The "palliative reflectance" study				The "hair cortical cell" project
	5 fraction	**10 fraction**	**12 fraction**	**20 fraction**	**32 fraction**
n	12	25	14	14	52
Male/Female	7/5	18/7	11/3	9/5	52/0
Age (years)	67.5(46-84)	62(40-84)	62.5(23-75)	65(42-80)	71(57-77)
Ant Chest	5	9	9	9	0
Post Chest	1	1	0	0	0
Abdomen	1	2	3	4	52
Lumbar-Sacral	5	12	2	1	0
Other	0	2	0	0	0
Heavy sun exposure Y/N	4/8	12/13	5/9	5/9	0/52
Fully bolused areas: **Total dose (Gy)**	22.1(17-26.1)	31.3(25.9-34.2)	40.4(30.6-44.4)	47.2(37-56)	-
Dose per fraction (Gy)	4.4(3.6-5.2)	3.1(2.6-3.4)	3.4(2.5-3.7)	2.4(1.8-2.8)	-
Unbolused areas: **Total dose (Gy)**	7.2(4.3-9)	7.5(4.4-15.9)	14.4(7.2-18.4)	18.9(8.2-25.1)	13.44(0.42-16.32)
Dose per fraction (Gy)	1.4(0.8-1.8)	0.75(0.4-1.59)	1.2(0.6-1.5)	0.95(0.4-1.3)	0.42(0.01-0.51)
Total field size (cm^2)	121(49-400)	159(66-1495)	124(81-539)	125(49-578)	75(54-105)
Overall time (days)	8(5-27)	15(12-20)	19(15-36)	30(28-33)	50(36-64)

Specified are median values (ranges) and absolute patient numbers

Table 1 Case composition of the two study groups

FIGURE LEGENDS

Figure 1 Schematic representation of the field sub-division (total field area greater than 175 cm^2) into differentially bolused areas along with test and control points for reflectance readings in the "palliative reflectance" study.

Figure 2 Peak percentage change in erythema recorded in the four fractionation groups of the "palliative reflectance" study according to the total measured dose at the skin surface.

Figure 3 The time course of reactions inside of the treatment field grouped according to the number of fractions delivered.

Figure 4 The time course of "reactions" 3 centimetres outside the field in a "control" area grouped according to the number of fractions delivered.

Figure 5 Reciprocal dose (Fe) plots based on isoeffective doses for the five fractionation groups calculated from probit analysis. Two regression lines have been arbitrarily fitted through the data points. One which fits through the 5, 10 and 12 fraction groups extrapolates to an α/β of 8.5 Gy. The other which fits the 20 and 32 fraction groups extrapolates to -1.6 Gy.

Figure 6 De Boer plot [3] for isoeffective data from the two studies. The linear quadratic model would be satisfied if the data points were to lie on a straight line.

Figure 7a Percentage change per fractional dose (i.e. peak percentage change/number of fractions) according to dose per fraction for patients treated in both studies.

Figure 7b Percentage change in erythema per day during the evolution of reactions according to dose per fraction.

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Low dose rate teletherapy and tumour response

C S HAMILTON, MB, BS, FRACR
S A SIMPSON
S FERGUSON
P OSTWALD
W HSU
M O'BRIEN
J W DENHAM, MD, FRCR, FRACR
Department of Radiation Oncology
Newcastle Mater Misericordiae Hospital, Waratah, NSW 2298, Australia

SUMMARY

Tumour responses in 25 patients with locally advanced head and neck cancer, treated on an experimental fractionated low dose rate (FLDR) teletherapy program are reported. Treatment was given at dose rates ranging from 1.8 to 3 Gy/h to a range of total doses from 32–38 Gy, with palliative intent. The total doses delivered have been predicted by the linear quadratic formula to be equivalent to 33–41 Gy using conventionally fractionated high dose rate treatment, in terms of acute normal tissue effects. A complete response rate (no visible or palpable disease 2 months after treatment) was observed in 28% of cases. Analysis of these response rates suggests that the linear quadratic formula may underestimate the anti-tumour effect of FLDR teletherapy at the various dose rates and total dose permutations in this study.

INTRODUCTION

There has been considerable debate in the world of clinical radiobiology as to whether low dose rate (LDR) radiotherapy is intrinsically associated with a therapeutic gain in considering normal tissue effect and tumour cell kill. Complicating factors such as the smaller treatment volumes and heterogeneous dose and dose rate distributions typically seen in clinical brachytherapy have made direct comparison of treatments at various dose rates very difficult. Recent evidence from laboratory studies, clinical studies and biomathematical considerations suggests that lowering of the dose rate itself may well be responsible for a therapeutic differential.[1–8]

Data on normal tissue damage end-points and tumour control rates for dose rates less than 1 cGy/min have been derived from extensive clinical brachytherapy experience. However the intermediate dose range between 1 cGy/min and 50 cGy/min has had less attention. In particular, in terms of external beam teletherapy, only a small number of groups have reported the effects of this modality on tumour response rates and/or normal tissue effects.[6–10] An experimental fractionated low dose rate (FLDR) teletherapy project was designed with the aim of accurately assessing acute normal tissue end-points in terms of mucosal and skin responses in an effort to provide systematic iso-effect data for a range of total doses and dose rates using external beam teletherapy from a Caesium-137 teletherapy unit.[1] Up to the present time patients have been treated at 1.8 and 3.0 Gy per hour, to total doses that have been predicted by the linear quadratic formula to be approximately equivalent in terms of acute normal tissue responses to 33–41 Gy using fractionated high dose rates (FHDR).[11] This study was not primarily designed to analyse tumour response rates, as it was recognized that patients with incurable locally advanced head and neck cancer would comprise a relatively heterogeneous population with limited life expectancy and were treated with purely palliative intent. It was not anticipated that any complete responses would be seen and therefore skin and mucosal reactions and palliation were chosen as the primary end-points for the study. However, since the project commenced, a number of very impressive tumour responses have been observed. We have therefore decided to prospectively examine these in detail and will reserve future reports for final analysis of the normal tissue end-points for the various permutations of dose and dose rate used.[1]

METHODS

Patients reported on in this interim analysis were treated on an experimental FLDR teletherapy programme at the Newcastle Mater Misericordiae Hospital. All patients were treated with palliative intent and selection criteria for consenting patients are given in Table 1. There were five T_4N_0, one T_3N_1, one T_3N_2, one T_4N_2 and 10 T_4N_3 patients entered on this study and considered by the authors to have incurable disease (seven patients presented with recurrent disease post-surgery).[12] Patients were treated to total doses of: (i) 32 Gy at 3 Gy/h (n = 8); (ii) 36 Gy at 3 Gy/h (n = 8); and (iii) 38 Gy at 1.8 Gy/h (n = 9).

Patients were treated on a Caesium teletherapy unit, operating at extended skin, source distance, designed to deliver treatment at the dose rate specified. Full technical and set-up details are given in a previous publication.[1] Patients were treated in a total of 10 daily fractions, with right and left lateral opposing fields and the dose was specified to the mid-point. All surface doses

Key words: dose rate effect; head and neck cancer; incomplete repair model; linear quadratic model; low dose rate teletherapy; palliative treatment.

Correspondence: Dr CS Hamilton, Department of Radiation Oncology, Newcastle Mater Misericordiae Hospital, Waratah, NSW 2298, Australia.

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were confirmed with thermo-luminescent dosimetry. Patients were reviewed twice weekly while on treatment for scoring of skin and mucosal reactions and bi-dimensional measurement of assessable disease. Response rates were assessed in the usual manner, with a complete response being defined as the absence of palpable and/or visual disease, at 2 months post-treatment and maintained for a minimum of 4 weeks; a partial response being > 50% decrease in the bi-dimensional product of the tumour; stable disease being < 50% increase in the bi-dimensional product; and progressive disease being > 25% increase in the bi-dimensional product. All patients were followed up after treatment at 2–4 weekly intervals for identical end-point assessment.

RESULTS

Seven of the 25 patients (28%) had a complete response to therapy. Fifteen patients (60%) had various other combinations of partial or complete response at the primary and/or neck site. The remaining 12% of patients had stable disease at one or both sites. Complete responses were maintained on average for a period of 75 days, and the average duration of complete relief of symptoms was 109 days. A complete response rate of 28% (7/25) at 2 months post-treatment has been maintained in two patients (each 300+ days). Four patients received further radiotherapy to the head and neck region with conventional external beam, 2–6 months following FLDR. One of these patients was classified as a complete responder to FLDR prior to this additional treatment.

Table 1 Patient selection criteria

1. Patients with squamous carcinoma of the head and neck that fulfil the RTOG modification of the American Joint Committee stage grouping criteria in the categories IVB, C, D and V excepting oral cavity tumours and laryngeal tumours that may be amenable to surgery.
2. Patients under 80 years of age with an anticipated survival of more than 8 weeks.
3. Patients who do not have intercurrent illnesses such as dementia, severe epilepsy, incontinence and severe musculo-skeletal disorders that are likely to make treatment lasting several hours each day difficult.
4. Patients who do not have severe dental caries requiring major dental work prior to treatment.
5. No prior treatment (radiation or chemotherapy).
6. Written informed consent to treatment on protocol.

Theoretical predictions

Application of Dale's modification of the linear quadratic formula to the total doses and dose rates used in this study reveals that doses of 32, 36 and 38 Gy at 3, 3 and 1.8 Gy/h respectively, are equivalent to 33–41 Gy using FHDR (2 Gy/fraction, 5 fractions/week). These figures are derived assuming a tumour α/β ratio equal to 25, and a repair constant (μ) equal to 0.69 $h.^{-1}$ This approach has neglected tumour proliferation and has assumed complete repair between the FLDR treatments (see Appendix I).[11]

The radiation therapy oncology group (RTOG) has published an algorithm derived from a logistic regression analysis for various head and neck cancer sites, based on one of their large head and neck protocol databases.[13] This algorithm uses a standardized coding for primary site, tumour stage and Karnofsky status, to calculate a probability of complete response 3 months after therapy. If this algorithm is applied to the present group of patients, then a 51% probability of complete tumour clearance is expected using conventional FHDR to a median dose of 65–68 Gy as employed by the RTOG. Four the 25 patients in this study are unsuitable for inclusion in this algorithm due to their having head and neck primary sites not applicable to the RTOG database. Figure 1 demonstrates the observed probability of complete tumour clearance in the head and neck group, plotted on the same graphic representation as RTOG database predictions, with two other data sets superimposed for comparison. The tumour control curves from Deacon represent nasopharynx and tonsillar data,[14] and the curve from Trott is based on tumour control data from carcinoma of the larynx.[15] In this representation the FLDR data from this study are expressed in terms of dose normalized to conventional FHDR.

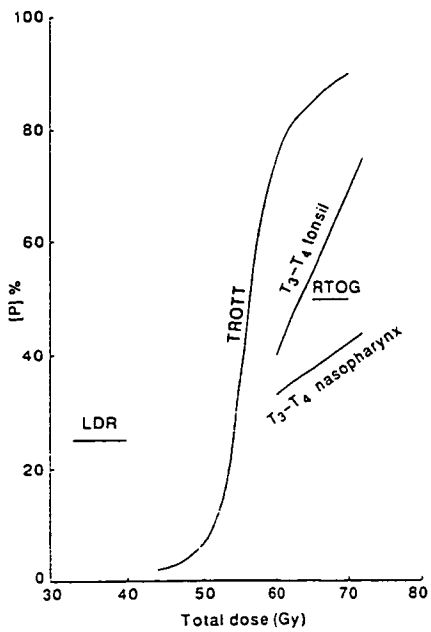

Fig. 1 Local control probabilities *vs* dose for this series (LDR), Griffin *et al.*,[13] Deacon[14] and Trott.[15]

DISCUSSION

The primary aim of this project was to test the predictions of the linear quadratic formula in the intermediate dose range (in terms of acute normal tissue toxicity). However it has become obvious to the treating clinicians that a higher percentage of complete responses than would have been predicted simply from mathematically equivalent total doses, or even from radiobiologically equivalent doses, has been seen. This is demonstrated in Figure 1, which suggests by extrapolation that virtually no T_3–T_4 head and neck tumour would be expected to respond completely to doses between 33 and 41 Gy (FHDR). The series from Deacon[14] and Trott,[15] of course, use varying definitions for local control at different time points. The RTOG experience, however, uses complete tumour clearance at 3 months as its basic reference. This might suggest that the biological activity of the physical doses used for the present study is, in fact, considerably higher than the linear quadratic formula predicts. Using the linear quadratic approach, of course, requires some assumptions regarding the most appropriate α/β ratio to use for squamous cell carcinoma of the head and neck, and the most appropriate repair half-time and repair model.[16,17] A tumour α/β ratio of 25 Gy was nominally chosen, although the choice of a lower value would not significantly alter the predicted equivalent doses. In the present example we elected to neglect the effect of tumour cell proliferation, and current evidence suggests that this is reasonable for a total treatment duration of only 12–14 days. It is

quite possible that the observed increase in biological effect may be partly due to the shorter overall time used in this study. Current evidence suggests that head and neck cancer may exhibit repopulation rates in the order of 0.4–0.7 Gy/day[18,19] and therefore the FHDR doses used for comparative purposes should perhaps be some 5–8 Gy lower. This interim analysis regarding tumour control rates suggests the need for further data obtained from patients treated to higher total doses than were used in this study. Pierquin's study drew a similar conclusion as they used external beam teletherapy at low dose rates, to a total dose of approximately 4500 cGy, and observed a significantly higher complete response rate in the LDR arm.[6,10] This was at the cost of a significant number of late necroses and we would recommended that before further dose escalations are made, current radiobiological predictions should be examined in more detail, particularly in the area of dose modifications required in the intermediate dose range between low and high dose rates. Biological efficiency is expected to vary significantly in this range, but substantial changes in the therapeutic ratio are not anticipated.[20,21] In fact it is only at dose rates below 1 Gy/h that clinically useful improvements in the therapeutic ratio are expected. The decision to test higher doses should therefore await an analysis of the anti-tumour effects in patients treated at 0.8 Gy/h, as is planned in the completion of this present study. Obviously, if even greater anti-tumour effects were to be observed at the same levels of normal tissue reaction observed at high dose rates, a firm case for testing high doses could be established.

The observed response rates to FLDR in this study must be viewed with caution as the patient numbers involved in this report are relatively small. Direct randomized comparison with an ostensibly equivalent FHDR treatment would be required to definitively establish tissue response equivalents for each regime. Survival prospects for many of the study patients were limited, and suggest the need to obtain systematic data at other body sites to further assess tumour response rates and durability of response.

The data also indicate that good symptom control may be achieved by this modality. It is not possible to speculate whether these palliative responses are sustained for a longer period than with FHDR.

ACKNOWLEDGEMENTS

This study was supported by grants from the Hunter Valley Cancer Appeal, New South Wales State Cancer Council and National Health and Medical Research Council.

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APPENDIX I

For fractionated low dose treatment we assume that repair is complete between fractions and that mono-exponential repair kinetics are followed.

For low-intermediate dose rates Dale[11] has shown:

$$RE = 1 + \frac{2R}{\mu(\alpha/\beta)}\left\{1 - \frac{1}{\mu T}(1 - e^{-\mu T})\right\} \quad 1$$

where RE is the relative biological effectiveness, R is the dose rate (Gy/h) and α/β is the ratio of single to multi-hit killing (Gy^{-1}).

In analogous manner, for fractionated high dose treatment the RE is given as:

$$RE = 1 + d/(\alpha/\beta) \quad 2$$

where d is the dose per fraction (Gy).

Iso-effective doses for the FHDR and FLDR regimes may be obtained by equating the extrapolated response doses (ERD) of each:

$$ERD_{FHDR} \times D_{FHDR} \quad 3$$
$$ERD_{FLDR} \times D_{FLDR} \quad 4$$

By substituting equations 1 and 2 into 3 and 4, respectively, it is trivial to obtain total dose FHDR equivalents.

Note that Nilsson *et al.* used an alternative approach utilizing the incomplete repair modification of the LQ model to obtain almost identical predictions (HD Thames, pers. comm.).[8]

The Radiographer 1992, 39: 156-157

A MOVEMENT DETECTION SYSTEM FOR PATIENT MONITORING IN LOW DOSE RATE TELETHERAPY

S. J. Howlett, J. W. Denham, S. A. Simpson, D. Pomare, D. Schmiedeberg, W. Dredge, R. Sadler and P. Hanbury

ABSTRACT

In an attempt to establish isoeffective conditions between the low and high dose rate ranges for human acute skin and mucosal reactions using teletherapy equipment, the Department of Radiation Oncology, Newcastle Mater Misericordiae Hospital, is currently conducting a Low Dose Rate (LDR) teletherapy program on a suitably modified caesium unit. In the course of treatment, maintaining patient position is crucial to an accurately delivered dose. This paper describes the development and use of an "in-house" movement detection system to achieve this goal.

Introduction

For this program patients are treated either semi-reclining in a modified dental chair or lying on a mobile treatment couch. In head and neck cases some immobilisation is achieved with customised polystyrene headrests. Nonetheless, since patients are required to maintain a fixed position over periods in excess of one hour movement will occur and so patients must be carefully monitored. Prior to the introduction of the proximity detector, patient movement was detected by the treatment radiographer monitoring a closed circuit television with a relevant outline of the patient drawn on the screen. Such a method has obvious shortcomings and it was felt that a reliable automated system should be in use. Through a combined staff input the department has achieved such a system.

System Description

The unit consists of three infra-red photoelectric proximity switches mounted on adjustable arms so as to detect movement in three separate directions. The arms are connected to a mobile support and so the unit is readily adjustable to individual treatments. (Fig. 1). The switches are wired to a control box mounted on a wall of the treatment room for easy viewing by both radiographer and patient. LED indicators on the control box display high, in, and low ranges for each channel in response to preset distance parameters. From the control box the signal is transmitted to the operator console where an in/out indication is given. Once the system indicates out of range in any direction there is a set time delay before the teletherapy machine is automatically switched off. Length of time delay along with the scanning range is adjustable (see Fig. 2 for specifications).

Development

The detector is the result of a combination of ideas and efforts from various divisions of the hospital staff namely clinicians, physics, electronics, radiography and mechanical. This combination resulted in requirements being matched by capabilities. Some time was required in the manufacture of a suitable mobile support and in the remission tests of suitable target material. No similar device is known to be in use in the radiation oncology area. While the idea and basic design is simple, the end result is a robust and reliable movement detection system.

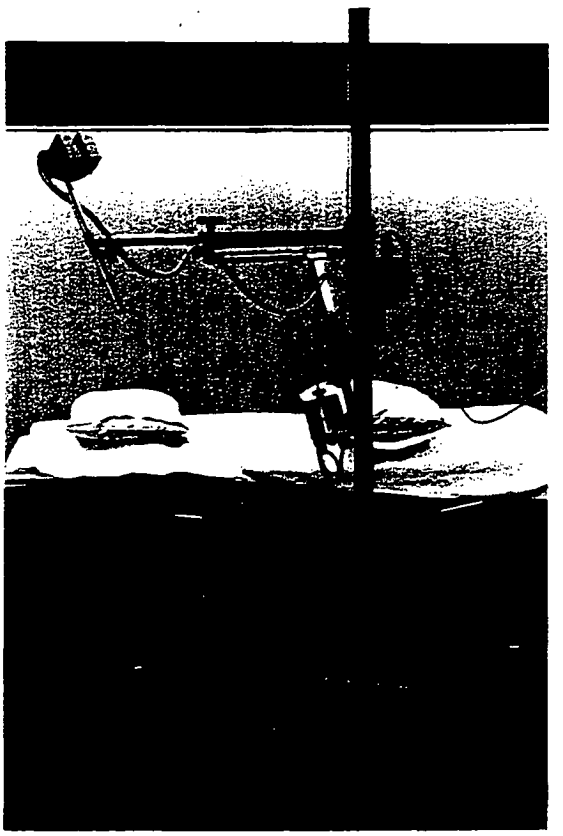


Fig. 1: The Movement Detection System.

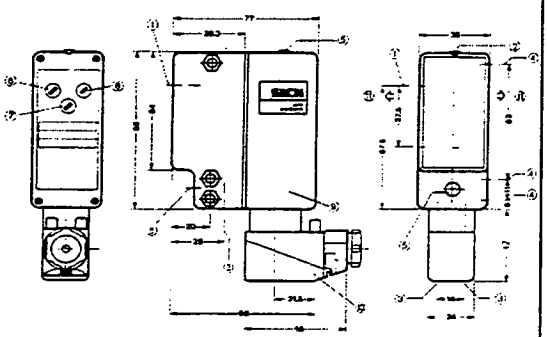
Set up Procedure

Normal treatment requires patient positioning by the radiographer in either chair or bed, correctly aligned with the caesium unit. Once this is achieved the detector is placed conveniently so that the three switches can reflect off the target, which has been placed in a position close to the field. (Fig. 3). The target is a thin white perspex circle

S. J. Howlett, J. W. Denham, S. A. Simpson, D. Pomare, D. Schmiedeberg, W. Dredge, R. Saddler, P. Hanbury.
Department of Radiation Oncology
Newcastle Mater Misericordiae Hospital.
Address for correspondence:
Mr. S. J. Howlett
Department of Radiation Oncology
Newcastle Mater Misericordiae Hospital
Waratah NSW 2298

of 3 cm diameter mounted on a raised cylindrical piece of clear perspex. It is raised in order to avoid reflections off the skin causing interference. Once the target is positioned, usually by double sided tape or similar, the detector arms are aligned by the use of a 50 cm pointer. This is the preset scanning distance with a tolerance set at ± 1 cm. Any movement outside of this tolerance causes the unit to switch off after a 30 second delay. In the case of head and neck patients a special cloth cap with velcro is used to allow placement of the target.

Scanning range	**200 to 800 mm, adjustable**
Supply voltage	**10 to 30 VDC**
Output	**NPN: Q + Q or PNP: Q + Q**
Time delays	**t_1, t_2, t_3, t_4**
Enclosure type	**IP 65 (dusttight, waterproof**
Type of light	**infrared**



① Middle of the optical axis
② Alignment sight
③ Threaded mounting hole M 5, 5.5 deep
④ Mounting holes through enclosure, recessed both sides for M 5 hex nut
⑤ Signal strength indicators
⑥ Device function switch
⑦ Scanning distance adjustment
⑧ Time delay control
⑨ 7-pin cable connector (for cables Ø 5 to 11 mm)
⑩ Cable receptacle (not included, see accessories)
⑪ Traveling direction of the material

All dimensions in mm (1 inch = 25.4 mm)

Fig. 2: Specifications of the SICK SENSWICK photoelectric proximity switches utilised in the system. (P/No WT 36-N 710).

Fig. 3: The system set up to monitor patient movement.

Performance

To date some 20 patients have had their treatments monitored by this system with the closed circuit television used as a back up. While some minor adjustments have been necessary, overall the system has been a success. In practice, it was found that only 2 sensors were required to detect adequately any movement outside the tolerances which led to an easier set up procedure. The system not only performed reliably but actually acted as an educating mechanism for patients regarding movement during treatment. Indeed, some patients monitored their own movement by observing the panel indicator lights and making appropriate adjustments to their position within the 30 second time delay thus avoiding breaks in treatment.

Conclusion

The development of the movement detection system for LDR teletherapy at this department is an innovation which has proved successful in improving the quality of treatment to LDR patients. It is the result of a combined staff effort directed at achieving this improvement. The LDR program is anticipated to continue here for another 12 months and this system will form an integral part of the treatments delivered.

Paper submitted July 1992. Paper accepted September 1992. Peer reviewed.

APPENDIX

3

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ile: !NEWPAT.FRM    Line: 29  of 67   Col: 1      F5F6F7F8F9F10   ▸▸ COLOR
          REFLECTANCE SURVEY PALLIATIVE PATIENTS NEWPAT FORM

ient Name        :  [LASTNAME    ], [FIRSTNAME    ]          (day/month/year)
ical Record      :  [MRNO        ]  Date Start Treatment   :  [DA_START  ]
                                    Date Finish Treatment  :  [DA_FIN    ]
                                    Overall Time           :  [OT        ]
                 :  [SEX       ]    Male/Female
                 :  [age]
tocol ID         :  [PROT_ID   ]       OESOPHAGUS/H&NCARBO/BREASTSYN/LDR:H&N
                                       /LDR:SKIN/PALREF/CONTROL
e Treated        :  [SITE      ]       SCLAV/ANT.CHEST/POST.CHEST/LOWBACK/
                                       ABDO
ications (Text)  :  [MEDIC_1   ]    [MEDIC_2   ]   [MEDIC_3   ]   [MEDIC_4   ]
                 :  [MEDIC_5   ]
r Colour         :  [HAIR      ]    BLOND/RED/BROWN/BLACK/OTHER
Teenager
ural Tanning     :  [TAN       ]    B++++TO/B+++T+/B++T++/B+T+++/BOTO
torical UV Score:  [UV_SCORE  ]    NIL/MILD/MOD/SEVERE  0 - 3 HISTORY
or UV Exposure   :  [FACE_UV   ]    NIL/MILD/MOD/SEVERE  0 - 3 TWO OBSERVERS
Face
or UV Exposure   :  [FIELD_UV  ]    NIL/MILD/MOD/SEVERE  0 - 3 TWO OBSERVERS
Treated Area
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a Partial        :  [AREA_P    ]          "       "
 Exposure Controls Only          ;[SUNEXP]            NO/YES
thema Value 1    :  [ERYTH1    ]   Erythema Value Control 1    :  [ERYTHC1    ]
thema Value 2    :  [ERYTH2    ]   Erythema Value Control 2    :  [ERYTHC2    ]
thema Value 3    :  [ERYTH3    ]   Erythema Value Control 3    :  [ERYTHC3    ]

genation Value 1:  [OXYG1     ]   Oxygenation Value Control 1 :  [OXYGC1     ]
genation Value 2:  [OXYG2     ]   Oxygenation Value Control 2 :  [OXYGC2     ]
genation Value 3:  [OXYG3     ]   Oxygenation Value Control 3 :  [OXYGC3     ]

anin Value First:  [MEL_1     ]
anin Value Peak :  [MEL_P     ]
anin Value Last :  [MEL_L     ]

 Value Area 1    :  [TLD1      ]    DOSE(cGY.)PER FRACTION ON SKIN, NO BOLUS
 Value Area 2    :  [TLD2      ]      "                "      THIN BOLUS
 Value Area 3    :  [TLD3      ]      "                "     THICK BOLUS

. Skin Dose 1    :  [CALDOSE1  ]      "                "
. Skin Dose 2    :  [CALDOSE2  ]      "                "
. Skin Dose 3    :  [CALDOSE3  ]      "                "

ber of Fractions:  [NO_FRACT  ]    NUMBER OF FRACTIONS OF TREATMENT GIVEN
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e Rate           :  [D_RATE    ]

                    [dip    ]       bottom of dip in months

                      D6          D12          D18          D24
ations    Area1     [d61    ]   [d121    ]   [d181    ]   [d241    ]
          Area2     [d62    ]   [d122    ]   [d182    ]   [d242    ]
          Area3     [d63    ]   [d123    ]   [d183    ]   [d243    ]
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              RADIATION ONCOLOGY LOW DOSE RATE PROGRAM
                        NEW PATIENT FORM


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ical rec #   :  [MRNO        ]
e of birth   :  [BIRTHDAY    ]                Age      :  [AGE    ]
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tocol ID          :  [PROT_ID       ]        OESOPHAGUS/H & NCARBO/BREASTSYN
                                             /LDR:H&N/LDR:SKIN/PALLREF

e of start tmt    :  [DA_START      ]
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                    EXPLANATORY VARIABLES


our Site          :  [TU_SITE       ]        SKIN/LIP/ORAL CAVITY/HYPOPHARYNX/
                                             OROPHARYNX/LARYNX/NASOPHARYNX/
                                             SINUS/NASAL VESTIBULE/NECK/SCLAV/
                                             ANTCHEST/TSPINE/RIBS/LSSPINE/ABDO/
                                             GROIN/SHLDE
ile: !NEWPAT.FRM    Line: 49  of 110  Col: 1      F5F6F7F8F9F10   ▸▸ COLOR
n trt site        :  [SK_SITE       ]        FCE/NCK/SHLDR/ARM/ANT CH/PST CH/RIB
                                             ABDO/GROIN/C-SPN/T-SPN/L-SPN/SCRM/
                                             BTTCK/LEG/EAR/CLVCL/SCPLA/AX/OTHER
tology            :  [HISTO         ]        UNKNOWN/BCC/SCC/ADENO/UNDIFF
tage              :  [T_STAGE       ]        T0/1/2/3/4
tage              :  [N_STAGE       ]        N0/1/2/3
tage              :  [M_STAGE       ]        M0/1

aovsky performance status    :  [KAR_PER] 0 to 100
e Rate                       :  [DOSERATE ] GY/HR.
n Dose Total                 :  [SKINDOSE ] GY
n Dose Entry Total           :  [SK_DO_EN ] GY
n Dose Exit Total            :  [SK_DO_EX ] GY
measured Dose                :  [MEASDOSE ] GY
osa Dose Total               :  [MUC_DOSE ] GY
ld Area 90%                  :  [FLD_SIZE ] SQ. CM.
or heavy sun exposure        :  [SUNEXP   ] NO/YES
rall time                    :  [OT       ] DAYS


ERPOLATIVE DATA FOR PEAK LEVEL OF REACTION; PTTS WHO DO NOT REACH PEAK ARE
LUDED.
      Toxicity MMH criteria:
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INTERPOLATIVE DATA DERIVED FROM DISCRETE REACTION LEVELS

ANY MISSING VALUES OR NOT APPLICABLE VALUES, SET TO MISSING.

			MUCOSA		SKIN
s after starting tmt:	Grade	1A reached	[DAYS1AM]	|	[DAYS1AS]
Dose:	"	" "	[ds1m]	Gy	
	"	1B "		|	[DAYS1BS]
	"	2 "	[DAYS2M]	|	[DAYS2S]
Dose:	"	" "	[ds2m]	Gy	
	"	3 "	[DAYS3M]	|	[DAYS3S]
Dose:	"	" "	[ds3m]	Gy	
	"	4 "	[DAYS4M]	|	[DAYS4S]
ation of reaction at					
n specified level:	Grade	1A duration	[DUR1AM]	|	[DUR1AS]
	"	1B "		|	[DUR1BS]
	"	2 "	[DUR2M]	|	[DUR2S]
	"	3 "	[DUR3M]	|	[DUR3S]
	"	4 "	[DUR4M]	|	[DUR4S]

APPENDIX

4

ELSEVIER

Radiotherapy and Oncology 35 (1995) 129–137

RADIOTHERAPY & ONCOLOGY

Acute reaction parameters for human oropharyngeal mucosa

J.W. Denham*[a], C.S. Hamilton[a], S.A. Simpson[a], M.Y. O'Brien[a], P.M. Ostwald[a], T. Kron[a], K.B.G. Dear[b]

[a]*Radiation Oncology Department, Newcastle Mater Hospital, Waratah, NSW 2298, Australia*
[b]*Statistics Department, University of Newcastle, Callaghan, NSW 2308, Australia*

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Abstract

The purpose of this study was to determine the influence of changes in dose rate over the range 0.8–240 Gy/h on acute oropharyngeal mucosal reactions in human subjects, and to estimate the values of the important parameters that influence these reactions. Sixty-one patients requiring radiotherapy to palliate incurable head and neck cancer were treated on a telecaesium unit, using opposing lateral portals to total midline doses, varying between 30 and 42 Gy in 10 daily fractions over 2 weeks, at dose rates of 0.8, 1.8, 3.0 and 240 Gy/h according to a central composite study design. The severity and time course of reactions were charted at least twice weekly for each patient, using the EORTC/RTOG acute mucosal reaction grading system. Duration of reaction at each grade was observed to provide a more sensitive reflection of effect than the proportion of patients reaching any particular reaction grade. Analysis of duration by direct and indirect methods suggest α/β ratios in the range 7–10 Gy and half-time ($t_{1/2}$) values in the range 0.27–0.5 h, if mono-exponential repair kinetics are assumed. The $t_{1/2}$ values are short and raise the question as to whether the repair kinetics of this tissue are well described by a mono-exponential function. Further prospective studies involving multiple daily fraction treatment regimes delivered at high dose rate, in which interfraction interval is deliberately varied, are needed to find out whether the parameters derived from this project are applicable to fractionated treatment courses at high dose rate.

Keywords: Mucosa; Low dose rate; High dose rate; Alpha/beta ratio; Repair kinetics

1. Introduction

Over recent years, considerable progress has taken place towards the development of models that will be helpful to clinicians in estimating the effects that are likely to be observed for many commonly used permutations of total dose, fractionation, and dose rate. The incomplete repair model, for example, provides very reasonable fits to many sets of laboratory and clinical data [23,24]. Of course, few clinical data are generated with the specific intention of defining the values of the parameters of the predictive models, therefore much of what is known is based on the re-analysis of historical clinical data [23]. In the past few years, increasing interest in the use of accelerated fractionation schedules delivering more than one treatment fraction per day has drawn attention to the importance of the rate at which repair occurs between fractions [10,14,15,18,28]. The process, which for operational purposes is considered, mono-exponential in the incomplete repair model [22], is best described by its half-time ($t_{1/2}$). Unfortunately, a dearth of relevant clinical data has prevented suitable values for this parameter from being derived with any certainty for any acute or late reacting human tissue. At the present time, therefore, a better understanding exists concerning appropriate values for α/β than for $t_{1/2}$ [23]. Increasing use of accelerated and hyperfractionated treatment regimes in locally advanced head and neck cancer has also drawn attention to the importance of the severity of acute mucosal reactions in determining treatment tolerance [18,25].

The fractionated low dose rate (FLDR) teletherapy project at the Mater Hospital, Newcastle, Australia [5],

* Corresponding author, Tel.: +61 (0)49 211177; Fax.: +61 (0)49 602566.

SSDI 0167-8140(95)01545-R

was therefore designed in response to the need for more prospective clinical data to describe α/β ratio, and $t_{1/2}$ parameters, for acute mucosal reactions as well as the dose rate effect itself. This has been done by examining reactions caused by identically fractionated treatment regimes, delivered at low to medium dose rates (i.e., under 10 Gy/h), and at high dose rate (240 Gy/h). This report describes the indirect and direct quantal analysis of these data and discusses the implications and remaining uncertainties of our results.

2. Patients, materials and methods

2.1. Patients

The 61 patients enrolled for treatment on this project had locally advanced squamous cancer of the head and neck (RTOG modification of AJCC criteria Stages IVC and V [16]), deemed incurable by any means, by the multidisciplinary consultative head and neck clinic of the Mater Hospital, and suitable for palliative treatment only. Also included were four patients with lesser stage disease, but concurrent incurable second primary cancers arising from other sites (two from lung, one from pancreas, one from cervix). Ineligibility criteria included severe intercurrent illness likely to result in failure to comply with the treatment and follow-up monitoring laid down below. Also ineligible were patients who had received prior treatment for head and neck cancer, including previous radiotherapy or chemotherapy. Informed, written consent to participate was the final prerequisite for entry. The study protocol was approved by the Hunter Area Research Ethics Committee in September 1988.

2.2. Treatment details

Technical details of the project have been published elsewhere [5]. Briefly, a Siemens Caesium teletherapy unit has been adapted to treat at 80 cm SSD, using specially designed rectangular collimating cones providing field sizes of between 80 and 168 cm^2 (median size used 100 cm^2). Adjustments to instantaneous dose rate has been achieved using lead filters. Treatments have been conducted using large opposing equally weighted lateral beams, with dose and instantaneous dose rate specified at the midplane point on the central axes. All 51 patients treated at low dose rate received 10-week daily treatment fractions with both fields treated on each treatment day. Beam direction has been achieved through the use from front and back pointers and verified using portal films. Dosimetry based on water phantom depth dose measurements for each individual treatment cone, has been verified during treatment using entrance and exit thermoluminescent dosimetry on the central axes of the beam. All treatments have been conducted with the patients sitting or semi-reclining in a dental chair, modified for comfort with a customised headrest to minimise movement of the head. In addition to the monitoring of patient movement by the operator (SAS) on closed circuit television, a specifically designed optical proximity switch was used as a failsafe mechanism to temporarily suspend treatment if a predetermined degree of excessive movement had taken place. The operation of this switch is reported elsewhere [13]. Rest breaks were allowed at frequent intervals during each daily treatment because the shortest treatment was 1 h 4 min per day in the patient group treated to 32 Gy total dose at 3 Gy/h.

The 10 patients treated at high dose rate on this project were treated using opposing lateral 6 MV beams on a Varian Clinac 1800 linear accelerator. Once again, all patients had 10 daily fractions with both fields treated each day and dose specified at the midplane point on the central axes of the beams. All treatments were conducted with the patient supine.

Anti-fungal prophylaxis was not administered to any of the patients.

2.3. Study design

As previously described [5], a modification to the central composite design concept has been used in this project, with the aim of determining isoeffective doses for 10 daily fractions delivered at 0.8, 1.8, 3.0 and 240 Gy/h (high dose rate). The central point of the composite design was 38 Gy at 1.8 Gy/h. The dosing levels at each dose rate selected have been structured around isoeffect predictions, using the incomplete repair model [7,17], and separated by intervals of approximately 10% in total absorbed dose. This decision was based in part on the need to establish the sensitivity of the endpoints used [8], and ensure adequate sample size. For logistic reasons, allocation to each group was determined consecutively (as opposed to random assignment). The final dosing groups are described in Table 1.

2.4. Study endpoints

Oropharyngeal mucosal reactions were graded at least twice weekly during treatment and after, until full recov-

Table 1
Details of 'dosing' groups used in the project; total dose and instantaneous dose rate is specified at the midplane in the central axes of the beam

Dose rate (Gy/h)	Total dose (Gy)	No. of patients	No. of fractions	Overall time (days)
240	30	10	10	12–14
3	32	10	10	12–14
3	36	9	10	12–14
1.8	38	10	10	12–14
0.8	36	7	10	12–14
0.8	38	6	10	12–14
0.8	42	9	10	12–14

Table 2
Mucosal grading criteria used in FLDR study (EORTC/RTOG toxicity criteria)

Mucous membrane	
0	No change over base line
I	Injection; mild pain not requiring analgesics
II	Patchy mucositis which may produce an inflammatory serosanguinous discharge; may experience moderate pain requiring analgesics
III	Confluent fibrinous mucositis; may include severe pain requiring narcotics
IV	Ulceration, haemorrhage or necrosis

ery had taken place using the EORTC/RTOG acute reaction grading criteria (Table 2). Often observations were obtained more frequently to better estimate the day on which the reaction changed from one grade to another. Time in days, from the start of treatment, to the onset and recovery from each reaction grade was recorded for each patient in addition to the peak grade reached. This enabled calculation of the duration of time that each patient spent at each reaction grade. Also measured were qualitative skin reactions using a modification of the EORTC/RTOG acute reaction grading criteria (Table 2), and reflectance spectrophotometry skin readings and tumour response rates reported elsewhere [9,12].

2.5. Analytical methods

All data were entered onto a temporal database (MEDLOG). Non-parametric exact tests such as the Fisher, Wilcoxon and Kruskal-Wallis tests, were performed using the StatXact software package. Direct estimates of α/β and $t_{1/2}$ were derived using the α/βest program [2]. Log likelihood based 'goodness of fit' probabilities were derived using a custom designed spreadsheet program on Lotus 1-2-3, while FLDR reciprocal dose plots [19], and other curve-fitting procedures were carried out using the Sigmaplot software package. Reciprocal dose plots were derived using the method used by Scalliet et al. [19], and described by Dale et al. [6]. A dose rate correction factor 'K' is applied to each dose rate to derive an equivalent fractional dose 'd'. Full details are given in Scalliet et al. [19].

3. Results

3.1. Time course of reactions

Complete data sets (i.e., days to onset, recovery and duration of each reaction grade) for 56 of the 61 patients were collected. Death due to intercurrent causes in four patients, (one from myocardial infarction; one from cerebrovascular accident; and two from pneumonia), and haemorrhage from the primary tumour in one patient occurred after treatment but prior to full recovery of all mucosal reactions, and therefore prevented calculation of reduction durations at all grades in the remaining five patients. In Table 3, median times for each group of patients to reach and recover from each reaction grade are presented. It will be noted that median time to recovery from each reaction grade increases as total dose increases. Data concerning the duration of time spent at each reaction grade for each group, together with the proportions in each group that reach each reaction grade, are summarised in Table 4. These data confirm the impression gained from Table 3 that reaction duration increases with total dose. Strong linear relationships were found to exist between the duration of time spent at any one reaction grade and another.

3.2. Isoeffect conditions

ED_{50} (0.5 probability of reaction duration reaching a specific value) estimates at each dose rate, have been de-

Table 3
Median times in days (range in parentheses) to the onset and recovery from reactions at Grades I, II and III level (the values with asterisks are derived from data obtained from less than five patients)

Group	Onset						Recovery					
	Grade I		Grade II		Grade III		Grade III		Grade II		Grade I	
	median	(range)	median	(range)	median	(range)	median	(range)	median	(range)	median	(range)
30 Gy @ 240/h	7.5	(6–9)	12.5	(9–16)	16	(11–23)	20	(16–31)	23	(13–33)	26.5	(18–46)
32 Gy @ 3/h	8	(2–14)	13	(11–18)	17*	(15–17)	19*	(18–20)	21.5	(17–28)	25	(17–33)
36 Gy @ 3/h	11	(4–12)	13.5	(9–16)	16.5*	(14–18)	21*	(18–28)	26	(21–31)	27.5	(16–37)
38 Gy @ 1.8/h	6.5	(3–11)	10.5	(7–15)	15.5	(10–22)	22	(17–30)	24	(21–37)	27.5	(24–44)
36 Gy @ 0.8/h	10.5	(5–14)	15	(6–17)	15*	(8–17)	17*	(17–20)	23.5	(21–30)	26	(23–37)
38 Gy @ 0.8/h	10	(5–13)	14	(7–16)	17.5	(12–22)	20	(18–24)	22.5	(16–27)	27	(21–37)
42 Gy @ 0.8/h	8	(5–10)	12	(7–16)	15	(9–18)	21	(18–24)	26	(19–31)	31	(26–36)

Table 4
Median times in days spent at each reaction grade; proportions reaching each grade are also provided

Group	*n*	Duration of time spent at:									Proportions reaching:			ED_{50} (Gy)
		Grade I			Grade II			Grade III			Grade I	Grade II	Grade III	
		n	median	(range)	*n*	median	(range)	*n*	median	(range)				
30 Gy @ 240/h	10	10	19	(11–37)	10	12	(3–17)	10	3.5	(0–8)	10/10	10/10	7/10	30
32 Gy @ 3/h	10	10	16.5[1]	(7–28)	10	8[1]	(0–15)	10	0[1]	(0–5)	10/10	9/10	3/10[1]	35.5
36 Gy @ 3/h	9	8	19[2]	(9–30)	8	12.5[2]	(0–16)	9	4[2]	(0–10)	9/9	8/9	6/9[2]	
38 Gy @ 1.8/h	10	10	22.5	(17–33)	10	14	(11–24)	10	2	(0–10)	10/10	10/10	7/10	36.5
36 Gy @ 0.8/h	6	6	14.5	(9–29)	6	8.5	(5–18)	6	1	(0–9)	6/6	6/6	3/6	
38 Gy @ 0.8/h	7	6	20	(11–25)	6	11.5	(2–16)	6	2.5	(0–8)	7/7	7/7	6/7	39
42 Gy @ 0.8/h	9	6	25.5[4]	(16–30)	7	13[4]	(11–29)	9	7[4]	(0–9)	9/9	9/9	6/8[4]	
Composite group:														
37 Gy @ 0.8/h (= 36 + 38 Gy groups)	13	12	19[3]	(9–29)	12	10[3]	(2–18)	12	1.5[3]	(0–9)	13/13	13/13	9/13[3]	
Wilcoxon 1-S Exact			1 vs. 2	0.27		1 vs. 2	0.028		1 vs. 2	0.03	Fisher Exact	1 vs. 2	0.128	
p values			3 vs. 4	0.04		3 vs. 4	0.086		3 vs. 4	0.21	*p* values	3 vs. 4	0.628	

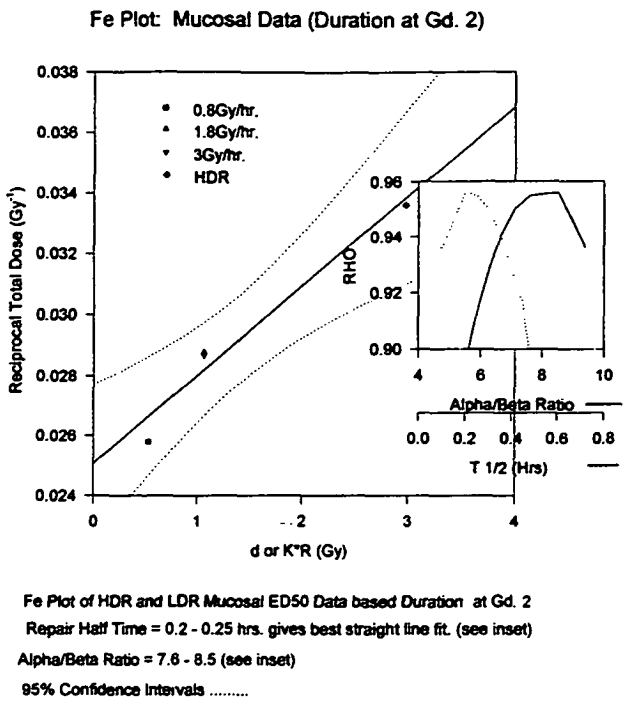

Fig. 1. Reciprocal dose plot for ED_{50} estimates for reaction durations at Grade II level. The inset graph describes correlation coefficient values (rho) for a straight line fit to the data points using various values of α/β and $t_{1/2}$. Best fit conditions are achieved by α/β values in the range 7.6–8.5 Gy and $t_{1/2}$ values in the range 0.2–0.25 h.

rived by interpolation from median and mean reaction durations at each reaction grade, as well as from the proportions in each treatment group whose reactions equal or exceed the median duration of reactions at each grade for the entire study population (Table 4). These estimates suggest that reactions to 30 Gy at 240 Gy/h are approximately equivalent to those caused by 35.5 Gy at 3 Gy/h (range 34.5–37 Gy), 36.5 Gy at 1.8 Gy/h (range 34.5–38 Gy), and 39 Gy at 0.8 Gy/h (range 38–40 Gy) at the three grades examined.

The ED_{50} estimates for Grade II reactions have been plotted on a modified reciprocal dose plot (Fig. 1). Best fit conditions are achieved by α/β values in the range 7.6–8.5 Gy, and $t_{1/2}$ in the range 0.2–0.25 h.

3.3. Direct quantal analysis, statistical uncertainty estimates

Direct quantal estimates of α/β and $t_{1/2}$, using the proportion of each group whose duration of reaction at each different reaction grade, equals or exceeds the median value obtained for the entire patient population combined, are provided in Table 5. Convergence on α/β estimates in the ranges α/β 6.7–10.4 Gy and $t_{1/2}$ 0.19–0.49 h are consistent with the results produced by the reciprocal dose plot methodology. It will be noted, however, that the 95% confidence intervals are quite wide, providing reason to explore the issue of statistical confidence further.

In Fig. 2, predictions of response proportions derived from the incomplete repair model are superimposed on the quantal responses actually observed in this project. It will be noted that while the α/β and $t_{1/2}$ parameters estimated for Grade II reactions (namely $\alpha/\beta = 8$, $t_{1/2} = 0.25$ h) provide a reasonable fit to the data, the parameters $\alpha/\beta = 15$, $t_{1/2} = 0.75$ h, provide a poor fit. In order to measure the goodness of fit of any given pair of parameter values to the observed data, the log likelihood ratio test for similarity between observed and expected binomial proportions was applied to a range of α/β and $t_{1/2}$ permutations, between α/β 5–15 Gy and $t_{1/2}$ 0.1–0.75 h. The results of this procedure for reactions at Grade II level are summarised in Fig. 3 and are in reasonable agreement with the direct analysis. This figure indicates that α/β values in the range 7.5–10 and $t_{1/2}$ in the range 0.2–0.55 h have a probability greater than 0.5 of fitting the data. Parameter values outside these ranges provide rapidly declining probabilities of fit, particularly for $t_{1/2}$ times below 0.2 h and α/β values above 15.

4. Discussion

An α/β estimate in the range 7–10 Gy, is entirely consistent with previous estimates [4,23]. The $t_{1/2}$ esti-

Table 5
Direct computations using the α/βest program of α/β and $t_{1/2}$ using the numerical proportion of patients in each group whose reaction durations equalled or exceeded the median duration of reactions at each reaction grade for the entire study population (the 95% CI for α/β and $t_{1/2}$ at Grades I and III are indefinite)

		α/β (95% CI)		$t_{1/2}$ (95% CI)	
Grade I	Logistic	10.3 Gy	(–)	0.18 h	(–)
	Poisson	10.4 Gy	(–)	0.19 h	(–)
Grade II	Logistic	9.0 Gy	(4.7–22.7)	0.24 h	(0.13–1.9)
	Poisson	9.3 Gy	(5.8–17.9)	0.25 h	(0.14–1.5)
Grade III	Logistic	6.6 Gy	(–)	0.43 h	(–)
	Poisson	6.7 Gy	(–)	0.49 h	(–)

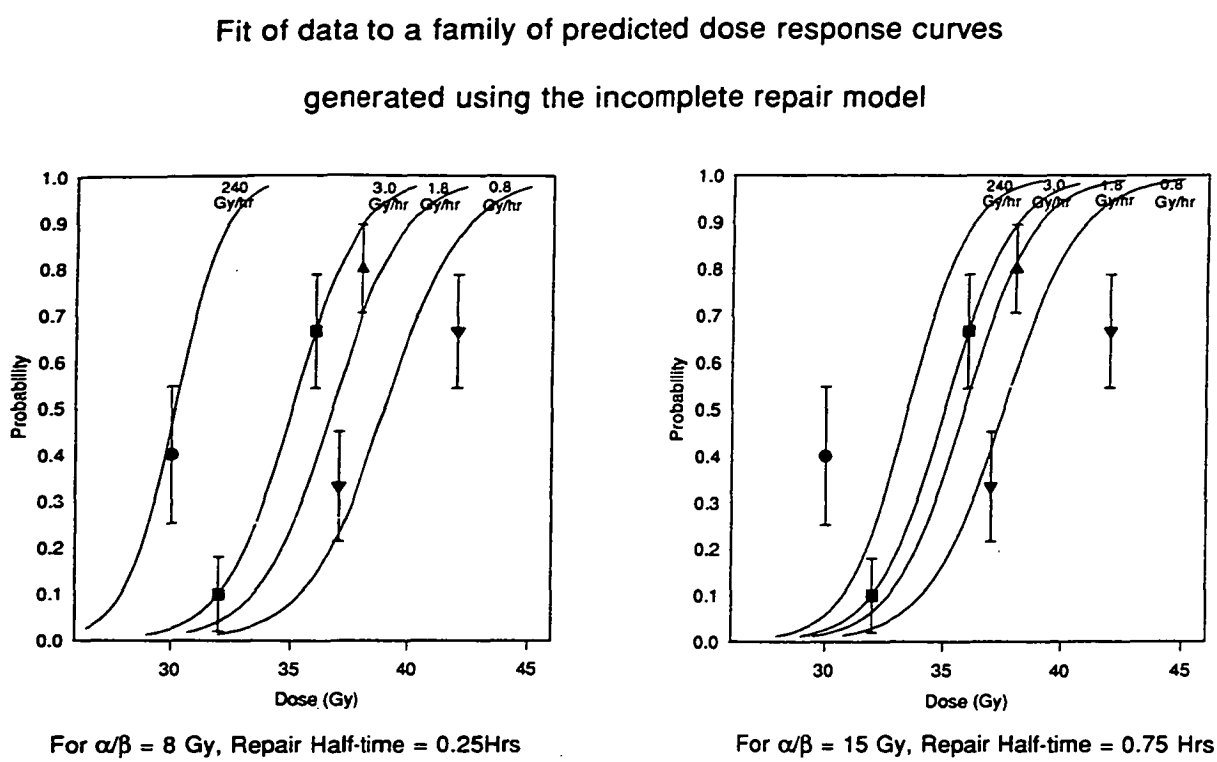

Fig. 2. Observed quantal responses superimposed on predicted response probability curves that were based on two different permutations of α/β and $t_{1/2}$ i.e., 8 Gy and 0.25 h and 15 Gy and 0.75 h. (N.B. The 'composite' group treated to 37 Gy at 0.8 Gy is formed from the groups treated to 36 and 38 Gy has been plotted.)

mate (range 0.25–0.5 h), however, is short and raises the question of the magnitude of uncertainties that may have impacted on the result.

These uncertainties can be addressed under three headings: technical aspects, the accuracy of the endpoints used, and sample size considerations.

4.1. Technical aspects

Turning first to the technical aspects it is clear that a number of factors could have impacted on dosimetric uncertainties. As discussed in an earlier report [5], the telecaesium beams used were not perfectly flat even at the 80-cm SSD used. Contour variations, scattering conditions and, above all, patient movement, provided additional uncertainties. Variation in overall treatment time is a factor that could have impacted on accumulated 'biological' dose, due to the possible onset of accelerated mucosal repopulation prior to treatment completion. Although it was intended the overall treatment duration would be 12 days, logistical difficulties prevented completion in less than 14 days in some patients. Although 'prolonged' treatments such as this did not affect any particular treatment group, it is a factor that may deserve revisitation as more becomes known of human mucosal repopulation kinetics.

4.2. Endpoints

Concerning the accuracy of the endpoints used, we have already drawn attention in a previous report to the fact that it is far easier to determine the level of a reaction on any particular examination day, than to estimate precisely how long a reaction at any particular grade lasts [8]. Factors involved include the matter of judgement that a reaction is changing from one grade to another on any particular day. Making this judgement more difficult is the fact that subsiding reaction appearances can be different to reaction appearances that are still evolving. Anatomical site differences, the presence of tumefactions and residual dentition are additional problems that we attempted to avoid by concentrating on the appearance of the oropharyngeal mucosa. Of the three reaction grade duration endpoints used in this study, we feel that duration at grade II has probably produced the most reliable α/β and $t_{1/2}$ estimates. Duration at the Grade I endpoint (erythema) is a difficult measure to judge reproducibly, and may not reflect a purely cytological endpoint [9]. Estimates based on duration at Grade III may be less reliable because only 37 of the 61 patients experienced this grade.

4.3. Sample size

Finally, the issue of sample size, which is one of the most important considerations in any study, is clearly an important one in this project. Uncertainties due to sample size perhaps dwarf other sources of error already mentioned. The wide confidence intervals around the re-

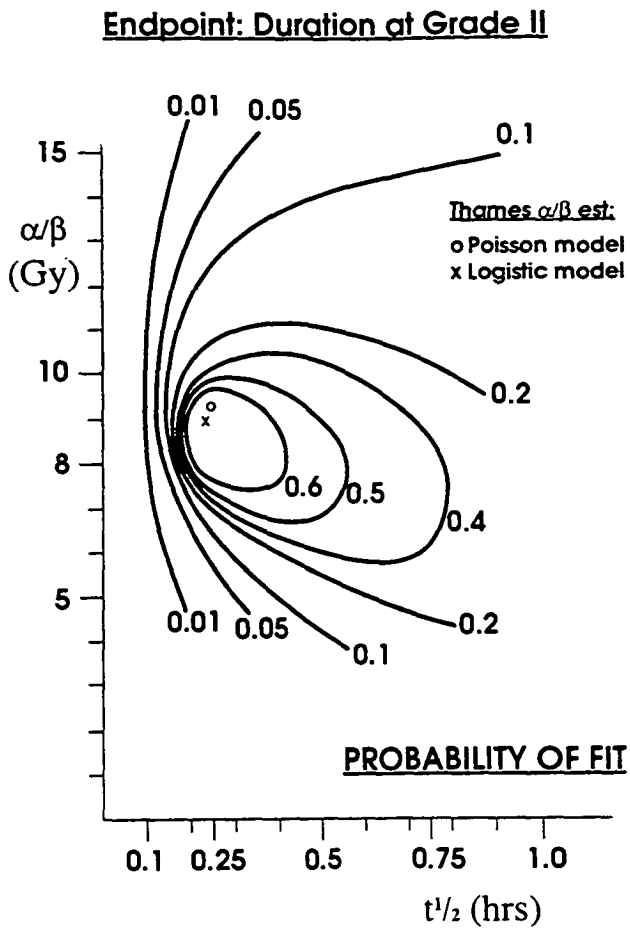

Fig. 3. A matrix of probability values that the observed data will fit various permutations of α/β and $t_{1/2}$ specified on the axes.

sults estimated by direct analysis (Table 5), and the diagram referring to goodness of fit to the data of a range of permutations of α/β and $t_{1/2}$ (Fig. 3), demonstrate this uncertainty quite clearly. Of course, these uncertainties are entirely due to the small numerical size of each treatment group. It is difficult to know whether a doubling of the sample size would have significantly increased the precision of our estimate, but this was not an option due to logistical and ethical constraints. While the logistical constraints of doing this are obvious, the ethical arguments are less straightforward. In any human volunteer study in which therapeutic benefits from the test treatment are not expected to exceed the benefit to be obtained from 'conventional' treatment [12] (i.e., fractionated palliative radiotherapy or chemotherapy), the ethical imperative is to discontinue the study after treatment of the minimum number of patients likely to produce a plausible result from the study. After we had become satisfied that a minimum of nine patients in each treatment group would allow us to resolve a difference in total physical dose of 10% [8] in our test system with all its uncertainties, we took the decision that the result obtained using groups of the stated size would satisfy these minimum conditions, i.e., be plausible.

Few published values for the α/β and $t_{1/2}$ values for mucosa are available for comparison with our results. Chougule and Supe recently reported α/β ratios in the range 7.68–8.11 Gy for acute mucosal reactions in patients with head and neck cancer treated with twice daily fractionation schedules [4]. Acute reactions in the mouse lip are one of the 'nearest' laboratory models. Various estimates of α/β have been obtained using this system. Ang et al. derived a value of 8.5 Gy using large fractional doses at high dose rate [1]. In a study employing a range of continuous low dose rate treatments, delivered at various dose rates, Scalliet et al. derived a value of 7.4 Gy [19]. In a subsequent study which investigated a range of interfraction intervals using fractionated high and low dose rate irradiations, Stuben et al. derived values in the 14.1–18.2 range [21]. Values for repair $t_{1/2}$ were in the 0.5–0.75 h range in the two sets of experiments that employed low dose rate irradiation [19,21], but were higher (0.9–1.2 h range) in the fractionated high dose rate study conducted by Ang et al. [1]. In mouse tongue mucosa, Dörr et al. derived an α/β ratio of 11.6 Gy and a $t_{1/2}$ value of 0.75 h in a study that involved high dose rate irradiation at various interfraction intervals [11]. Thames et al. concluded that human tissue repair $t_{1/2}$ values are usually slightly longer than those obtained in rodents [23]. However, the only value for the human mucosa published to date is 2 h or more, which grossly exceeds the mouse lip estimates [23]. It also exceeds the direct analysis estimates of 1.3 h for erythema and 1.1 h for desquamation in human skin obtained from Turesson and Notter's internal mammary irradiation data [26,27]. The 2-h estimate for the human mucosa was derived from data obtained in the randomised RTOG trial in advanced head and neck cancer, which compared a conventionally fractionated treatment of 66–74 Gy in 7–8 weeks, with the 60 Gy in 5 weeks utilising twice daily fractions of 1.2 Gy reported by Marcial et al. [15]. In this study interfraction interval ranged from 3 to 7 h. Both early and late reactions occurred more frequently in patients whose interfraction intervals were less than 4.5 h. 'Severe mucositis' occurred in 20 of 72 (28%) patients treated with interfraction intervals less than 4.5 h, whereas it occurred in only one of 20 (5%) of patients treated with longer intervals ($p = 0.06$). Since this was a multi-institution study, in which patients were not randomly assigned to specific interfraction intervals and treatment was interrupted in 36% of patients treated on the twice a day schedule, it is difficult to know what significance to attach to these observations. However, increased mucositis was also reported in a pilot study of concomitant boost therapy in Poland [3]. In this study the incidence of confluent mucositis during the concomitant boost phase of treat-

ment was reduced when interfraction interval was increased from 4 to 6 h. Steel et al. noted that in cell line experiments shorter $t_{1/2}$ estimates were obtained from dose rate analyses employing a range of low and intermediate dose rates, than from split dose experiments using high dose rate irradiation [20]. Dale et al. witnessed a similar phenomenon for dose rates up to 25 Gy/h in the C3H mouse jejunum [7], which added weight to the suggestion that repair may be a multicomponent process that is not always well described by a mono-exponential function. Perhaps this explanation provides some reconciliation between the long estimate obtained from the RTOG and Polish mucosal data, and the short estimate obtained in this project. It could be that repair of a rapid component of what is in fact a multi-exponential repair process in human mucosa is insignificant after irradiation at high dose rate, but is important during protracted exposure at low dose rate. If this is true then the incomplete repair variant of the linear quadratic model, which assumes mono-exponential repair kinetics, does not provide a perfect description of acute mucosal reactions under all conditions as it stands. It would also mean that parameters derived from dose rate studies, such as this one, should be applied with extreme caution to fractionated treatment courses delivered at high dose rates.

5. Conclusions

This project has produced data that provide α/β ratios of 7–10 Gy and $t_{1/2}$ values of 0.25–0.5 h for the acutely reacting human mucosa when the incomplete repair model is applied. The $t_{1/2}$ values are short, however, and raise the question as to whether the repair kinetics of this tissue are well described by a mono-exponential function. Further prospective studies involving multiple daily fraction treatment regimes delivered at high dose rate, in which interfraction interval is deliberately varied, are needed to know whether the parameters derived from this project are applicable to fractionated treatment courses at high dose rate.

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